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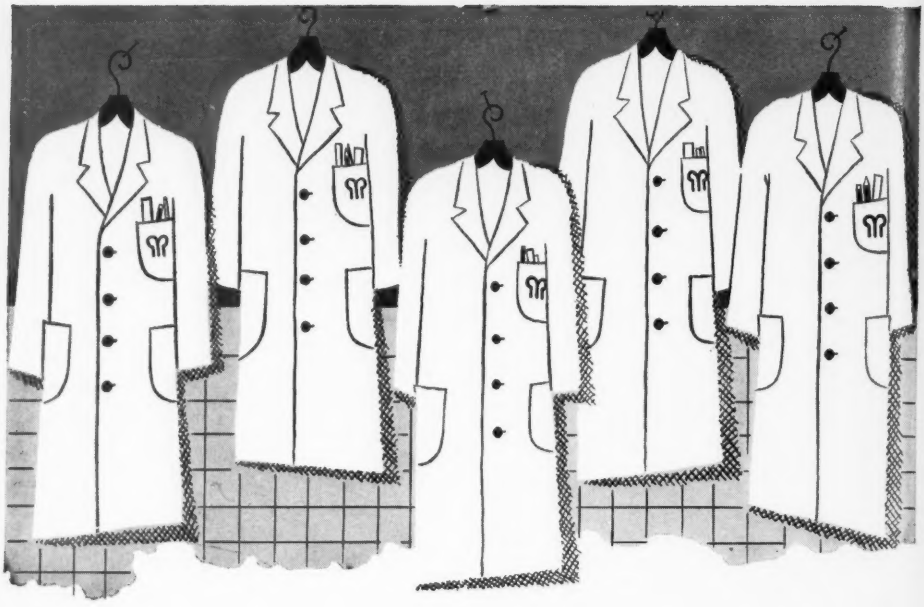

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THE EFFECT OF ADRENOCORTICOTROPIN ON THE EXCRETION OF ADRENAL METABOLITES IN NORMAL HUMAN SUBJECTS*

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IN CONJUNCTION with studies carried out by McAlpine *et al.* (1) on the effect of adrenocorticotropic hormone (ACTH) on carbohydrate and electrolyte metabolism in normal individuals, simultaneous studies on the excretion of adrenal metabolites were made.

Total 17-ketosteroids, β-OH ketosteroids, glucocorticoids, and reducing substances were determined before and after the administration of large doses of ACTH. In 3 cases the urinary metabolites were fractionated into ketonic and nonketonic water-soluble and benzene-soluble substances and the reducing power of these fractions was measured.

EXPERIMENTAL

The subjects used for this investigation were 5 normal adult men and 1 woman. In order to conduct balance studies, a constant diet was started in all but 1 subject (Case 2) and observations were made over a control period varying from two to seven days. Three diets were used, the daily caloric intake varying from 3,140 to 3,250 calories and the protein from 103 to 111 Gm. Each subject was maintained on the same diet throughout the study.

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ACTH[1] was administered intramuscularly in varying doses as described subsequently for the individual cases and the study was continued for two to seven days. Two different batches of ACTH were used in these studies, 37-KE which has a potency of 50 per cent of the Armour Standard LA-1-A, and 32D which has 70 per cent of the activity of the Standard LA-1-A. In the charts the dose of ACTH is expressed in terms of the Standard. Urine was collected in 12-hour or 24-hour periods and the excretion of the various adrenal metabolites was followed before and after the administration of ACTH. The values for the total amounts excreted in twenty-four hours are shown in Figures 1 through 5.

Methods

17-Ketosteroids: An aliquot of the urine was acidified with 15 volumes per cent of H_2SO and simultaneously hydrolysed and extracted by boiling with CCl_4 for three 2-hour periods. The ketonic fraction was separated by means of Girard's reagent T and the 17-ketosteroids measured colorimetrically by the method of Holtorff and Koch (2). The β-hydroxy-17-ketosteroids were determined directly by the micro method of Frame (3).

Glucocorticoids: The glucocorticoids were measured by the bioassay method of Venning, Kazmin and Bell (4) on adrenalectomized mice and are expressed in terms of glycogenic units, one glycogenic unit being equivalent to the biologic activity of one gamma of 17-hydroxy-11-dehydrocorticosterone. The total neutral corticoid fraction was used in this assay.

Reducing substances: Adrenocortical metabolites were also assayed by the chemical method of Talbot *et al.* (5). This determination is carried out on the water-soluble ketonic fraction and the results are expressed in terms of reducing power of 11-dehydrocorticosterone.

Fractionation of corticoids: The total neutral steroids obtained by extracting the unhydrolyzed urine with chloroform at pH_2 were dissolved in 30 cc. of benzene, and the benzene solution was extracted 10 times with 30 cc. of water. The water-soluble fraction was further separated into ketonic and nonketonic material by Girard's reagent T. In three of the studies the reducing properties of these various fractions were compared. Of the 6 normal subjects used in this study, 4 remained ambulatory (Cases 1, 2, 3, 4) and carried out their normal duties. The other 2 (Cases 5 and 6) were maintained in the metabolic ward.

Clinical Material

Case 1—H. M., male, age 35 years, weight 150 lbs., was started on a constant diet and was studied over a control period of four days. The urine was collected in 12-hour periods. 17-Ketosteroids, glucocorticoids, and reducing steroids were measured. On the fifth day a total of 120 mg. of ACTH (37-KE), or 60 mg. of the Armour Standard was administered in two doses over a period of one hour. The study was continued for five days. The values obtained for the various urinary metabolites are shown in Figure 1. The results are expressed in terms of amount excreted per twenty-four hours.

---

[1] The adrenocorticotropic hormone was generously supplied by Armour and Company, through the courtesy of Dr. John R. Mote.

Case 2—J. H., male, age 34 years, weight 160 lbs., was the subject of two experiments. In the first experiment, after a control period of two days, a total of 200 mg. of ACTH (32-D), or an equivalent of 140 mg. of the Armour Standard, was administered in six divided doses over a period of twenty-four hours. The study was continued for three days.

In the second experiment a total of 360 mg. of ACTH (37-KE), or 180 mg. of Armour Standard, was administered in six divided doses over a period of twenty-four hours and the various adrenal metabolites were followed for five days. The neutral corticoid fraction was separated into water-soluble ketonic and nonketonic substances, and benzene-soluble substances, and each fraction was measured separately for its reducing capacity. The 17-ketosteroids were partitioned into α and β hydroxy-ketosteroids. The results are charted in Figure 2.

Case 3—L. F., male, age 24 years, weight 150 lbs., received 420 mg. of (37-KE), or 210 mg. of Armour Standard, injected in seven divided doses over a period of twenty-four hours. This individual was studied for a period of seven days prior to the administration of ACTH and then for a further seven days after ACTH. Fractionation studies were also carried out in this experiment. The results are shown in Figure 3.

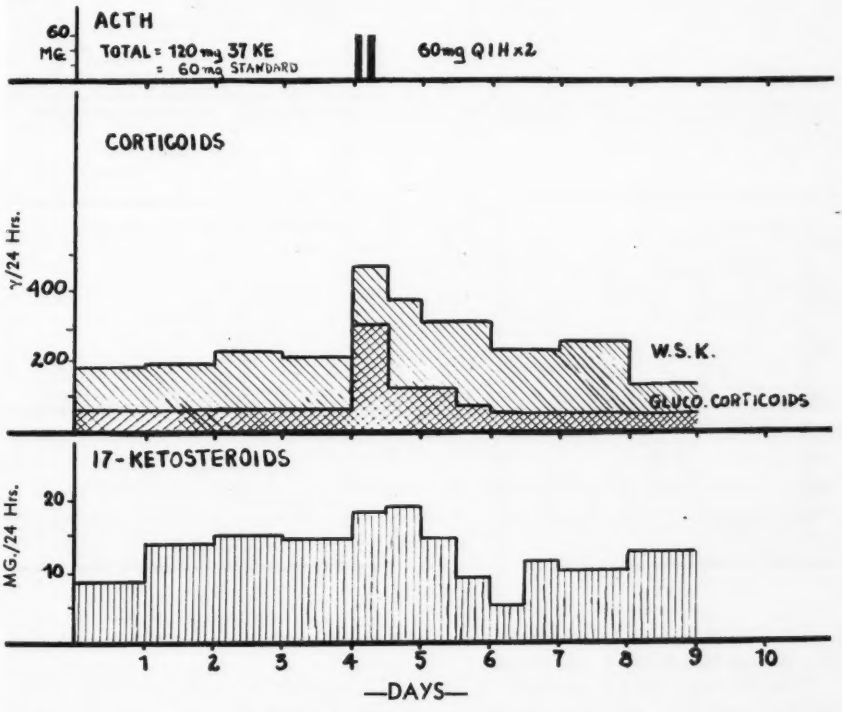

FIG. 1. *Effect of ACTH administration on the excretion of corticoids and 17-ketosteroids in a normal man (Case 1. Age 35 years.).*

W.S.K. = Reducing steroids in the water-soluble ketonic fraction.

Glucocorticoids are the biologically active steroids in the total neutral fraction.

Case 4—P. H., female, age 30 years, weight 134 lbs., was given 420 mg. of ACTH (37-KE), or 210 mg. of Armour Standard, in seven divided doses over a period of twenty-four hours. The urine was collected in 12-hour periods during and after the administration of ACTH. The changes in excretion rates of the various adrenal metabolites are shown in Figure 4.

Case 5—S. G., male, age 29 years, weight 205 lbs., received 480 mg. of ACTH (37-KE) (60 mg. every four hours for eight doses).

Case 6—D. S., male, age 22 years, weight 125 lbs., also received 480 mg. of ACTH (37-KE) (60 mg. every four hours for eight doses).

These last 2 subjects were studied for shorter periods of time. The results are charted in Figure 5.

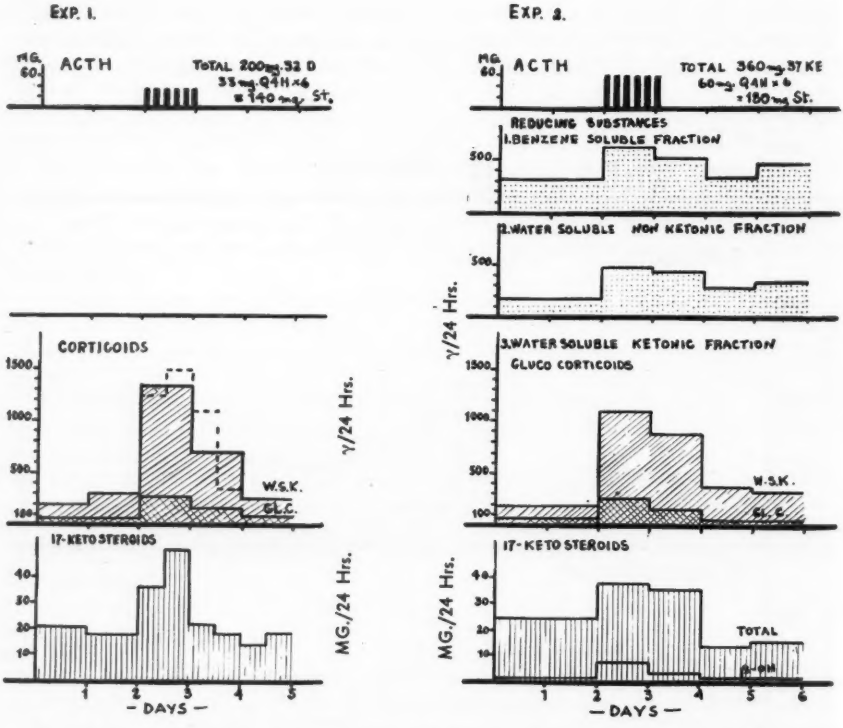

FIG. 2. *Effect of ACTH administration on the excretion of corticoids and 17-ketosteroids in a normal man (Case 2. Age 32 yrs.).*

The reducing steroids were determined in 3 fractions: the benzene-soluble, water-soluble nonketonic, and water-soluble ketonic fractions. The glucocorticoids were measured in the total neutral fraction only.

The dotted line represents the excretion rate of the reducing steroids in 12-hour specimens; the solid line, in a 24-hour specimen.

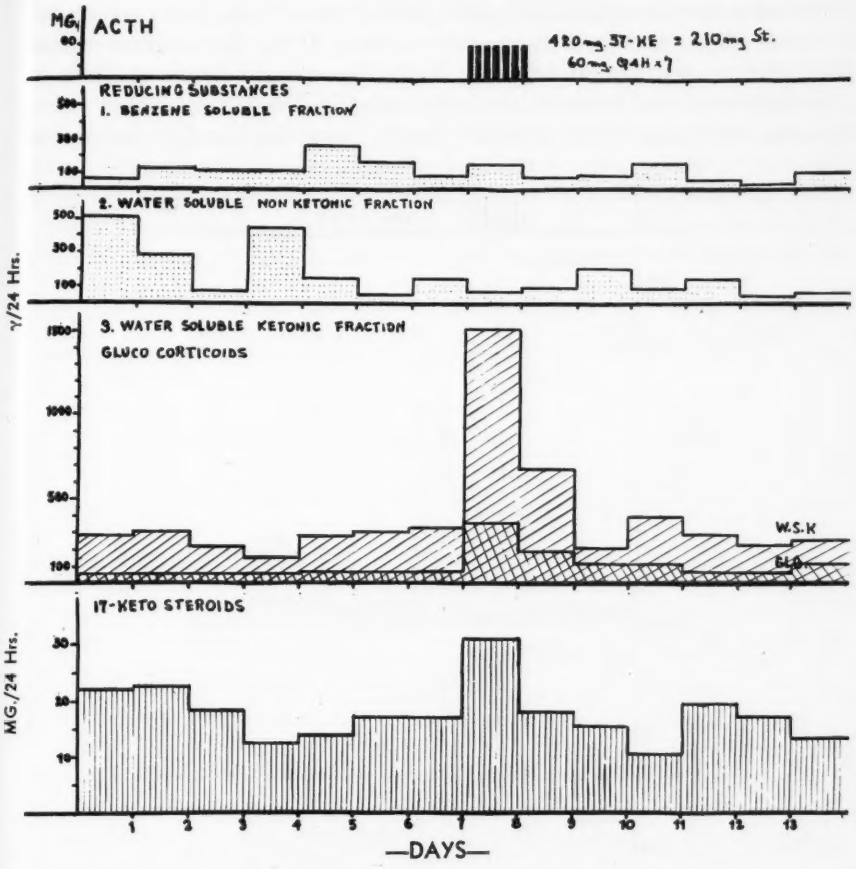

FIG. 3. *Effect of ACTH administration on the excretion of corticoids and 17-ketosteroids in a normal man (Case 3. Age 24 yrs.).*

The reducing steroids were determined in 3 fractions: the benzene-soluble, water-soluble nonketonic, and water-soluble ketonic fractions. The glucocorticoids were measured in the total neutral fraction only.

RESULTS

17-Ketosteroids

In all seven experiments there was an increase in output of 17-ketosteroids following the administration of ACTH. The patient (Case 1) who received the smallest dose of ACTH (120 mg.) showed the least effect. However, there was considerable variation in the response to the larger doses. The total increase in output in mg. per twenty-four hours on the first

day of administration of ACTH varied from 5.7 to 17.9 mg. When calculated in terms of percentage increase over control values, this increase varied from 43.5 per cent to 105 per cent. These changes are shown in Table 1.

In 3 of the normal subjects, the increased output in 17-ketosteroid excretion was maintained for forty-eight hours (Cases 2B, 4 and 6) whereas in

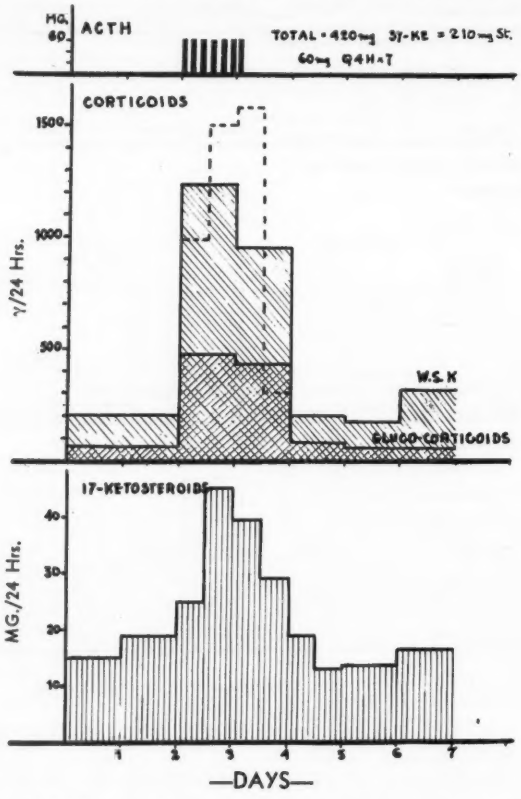

Fig. 4. *Effect of ACTH administration on the excretion of corticoids and 17-ketosteroids in a normal woman (Case 4. Age 30 yrs.).*

W.S.K. = Reducing steroids in the water-soluble ketonic fraction.

The dotted line represents the excretion rate of the reducing steroids in 12-hour specimens; the solid line, in a 24-hour specimen.

the remainder it was not maintained beyond the first 24-hour period, in spite of the fact that (with the exception of Case 1) the last injection of ACTH was given on the morning of the second day. In Case 6, in which instance the injections of ACTH were continued over into the second day, the 17-ketosteroids were still increasing. Unfortunately, these studies

could not be continued beyond the 48-hour period. In Case 5, in which similar amounts of ACTH were administered, there was no effect on the second day of ACTH therapy. With the exception of Case 6, in all the patients the values for 17-ketosteroid excretion were back to normal levels after forty-eight hours. In the 3 cases in which the total 17-ketosteroids were fractionated into α and β hydroxy-steroids, an increase in both these fractions occurred following ACTH administration. In Case 2A the excretion of β-hydroxy-steroids increased from 1.3 mg. to 7.4 mg. per twenty-four hours, and in Case 5 from 2.3 mg. to 6.2 mg. per twenty-four hours.

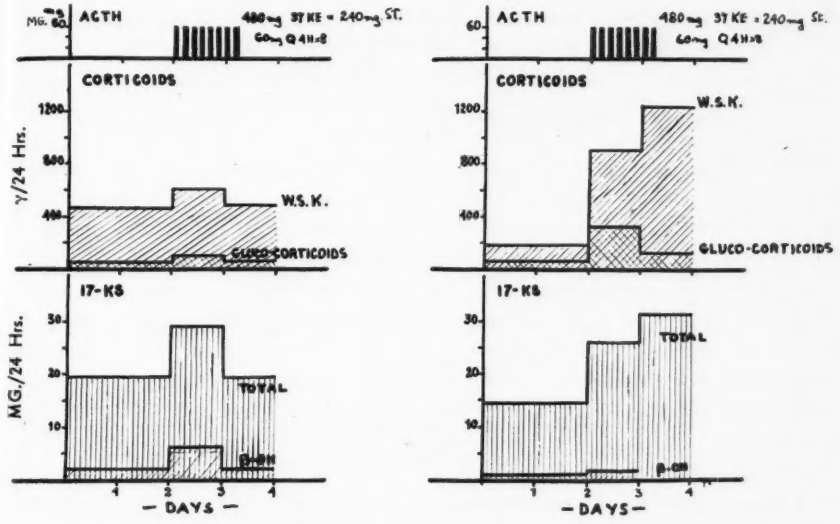

FIG. 5. *Effect of ACTH administration on the excretion of corticoids and 17-ketosteroids in 2 normal men (Case 5, left. Case 6, right.).*

W.S.K. = Reducing steroids in the water-soluble ketonic fraction.

In Case 6 the difference between the two periods was too small to be of any significance. In all 3 cases the ratio of β-hydroxy-ketosteroids to total 17-ketosteroids was greater after administration of ACTH.

Glucocorticoids

This term has been applied to those adrenal metabolites having the property of affecting carbohydrate metabolism. With the exception of Case 5, in all instances there was a marked increase in urinary glucocorticoids following the administration of ACTH. Within twelve hours after the first injection the excretion of glucocorticoids was above the control level, and in most cases the peak in excretion was reached in thirty-six

hours. Within twenty-four hours after the last dose the glucocorticoids were back to normal levels. The increases ranged from 258 to 565 per cent of the control level (Table 1). In Case 6 the study was not continued long enough after the last dose of ACTH to show the abrupt fall in excretion of glucocorticoids which usually occurs. In none of the subjects, when the studies were continued for an adequate period after ACTH administration, did the urinary glucocorticoids fall below the control levels. There was an impairment in carbohydrate tolerance in the 4 subjects receiving the large dose of ACTH and all showed glycosuria (1). The least effect was observed

TABLE 1. PERCENTAGE INCREASE IN THE EXCRETION RATE OF VARIOUS ADRENAL METABOLITES DURING THE FIRST TWENTY-FOUR HOURS AFTER ADMINISTRATION OF ACTH

Case No.	ACTH in terms of standard	17-Ketosteroids		Gluco-corticoids	Reducing steroids		
		Total	β OH		W.S.K.*	W.S.N.K.†	B.S.‡
	Dose in mg.	% increase	% increase	% increase	% increase	% increase	% increase
1	60	44	—	258	110	—	—
2A	140	132	—	320	360	—	—
2B	180	54	470	368	350	210	96
3	210	72	—	542	470	0	0
4	210	105	—	565	480	—	—
5	240	51	170	72	40	—	—
6	240	77	—	390	408	258	170

* Water-soluble ketonic fraction.
† Water-soluble nonketonic fraction.
‡ Benzene-soluble fraction.

in Subject S. G. (Case 5) in whom the increase in glucocorticoids did not exceed the upper limit of normal range. This man excreted a total of 7.3 Gm. of glucose following ACTH administration, whereas the other 3 subjects excreted 21 to 31 Gm. of glucose.

Reducing steroids

The assay of the reducing steroids was carried out on the water-soluble ketonic fraction and in general it was found that this assay paralleled the changes in the bioassay, although the total amount of reducing steroids was always greater than the amount of biologically active material. When fractionation studies were made, as in Cases 2B, 3 and 6, reducing steroids were also assayed in the two other fractions, the water-soluble nonketonic and the benzene-soluble fractions. The variations occurring in these last

two fractions fluctuated more widely. In Cases 2B and 6 an increase occurred in these fractions following ACTH administration, whereas in Case 3 there was no change. By far the greatest increase occurred in the water-soluble ketonic fraction.

DISCUSSION

As a comprehensive account of this investigation of the effects of ACTH on electrolyte, carbohydrate and nitrogen metabolism in normal individuals will be reported in a separate paper, these effects will be only briefly referred to here.

Administration of these relatively large doses of ACTH caused a striking change in the excretion of all of the recognized adrenal steroid metabolites. The 17-ketosteroids increased to levels well above the normal range, the maximum being 44 mg. per twenty-four hours. This effect could be observed within six to twelve hours after administration of ACTH. With lower doses both Mason *et al.* (6) and Forsham *et al.* (7) found that the 17-ketosteroids were not increased beyond the normal range. As increased excretion of β-OH ketosteroids, particularly dehydroisoandrosterone, has been associated with adrenal hyperactivity, it was interesting to note that the greatest increase in this fraction did not exceed 20 per cent of the total ketonic fraction. This increase falls within the range observed in cases of adrenal hyperplasia. In isolation studies carried out by Mason *et al.* (6), no dehydroisoandrosterone could be found before or after the administration of ACTH. In those subjects in whom the studies were continued for a sufficient time after hormone administration was stopped, withdrawal caused a decrease in 17-ketosteroids slightly below the control levels. Within four days the 17-ketosteroids had returned to normal levels. This effect was not observed with the glucocorticoids or with the reducing steroids. McAlpine *et al.* (1) showed that there was a marked retention of sodium and chloride and an increased excretion of potassium following ACTH administration in these patients and the typical "rebound phenomena" observed by Forsham *et al.* (7) occurred on withdrawal of the hormone.

The excretion of biologically active substances, the glucocorticoids, was greatly affected by the administration of ACTH. With 1 exception (male), these substances increased in the urine to amounts usually associated with Cushing's syndrome. The exceptional subject showed a rather poor response to ACTH as judged by changes in excretion of glucocorticoids and reducing steroids, and the increase in 17-ketosteroids was also less than that seen in the other 3 subjects receiving the large dose of ACTH. Although glycosuria did occur, it was much less severe than in the other 3 individuals. This man was much heavier than the others and was maintained on the same caloric intake. Whether marked differences in weight among indi-

viduals might account for the variations in response it is not possible to say. The increase in nitrogen excretion observed in these subjects was not sufficient to account for all the glucose excreted on the basis of gluconeogenesis alone, and a decreased ability to utilize carbohydrate must be considered (1). The corticoids, the water-soluble reducing substances, showed similar changes to the glucocorticoids and within six hours after the administration of ACTH a rise in these substances could be detected. An analysis of the various corticoid fractions revealed that the ketonic water-soluble fraction showed a greater response to ACTH than the nonketonic water-soluble and benzene-soluble fractions. In 1 case these last two fractions were not altered by ACTH, whereas in 2 other cases increases were observed. The ketonic water-soluble fraction contains a greater proportion of the more highly oxygenated adrenal steroids, including the active glucocorticoids.

SUMMARY

The administration of ACTH in doses ranging from 60 mg. to 210 mg. (Armour Standard LA-1-A) to 6 normal individuals caused an increase in the excretion of urinary steroids. The ACTH was given in divided doses over a period of twenty-four hours.

The total urinary neutral 17-ketosteroids increased from 44 per cent to 105 per cent of the control values during the period of administration of ACTH; β-OH-17-ketosteroids were also increased.

The glucocorticoids showed increases ranging from 72 per cent to 565 per cent; the corticoids (ketonic water-soluble reducing steroids), assayed chemically, from 40 per cent to 480 per cent.

Studies on the various corticoid fractions, the water-soluble ketonic, the water-soluble nonketonic, and the benzene-soluble fractions, showed that the greatest increase in excretion occurred in the water-soluble ketonic fraction. In 1 case no effect was observed in the benzene-soluble and water-soluble nonketonic fractions, whereas in 2 cases there was an increase in these fractions.

Within six hours after administration of ACTH an effect on the steroid excretion could be observed. In most of the cases the urinary steroids had returned to control levels twenty-four hours following the last administration of ACTH.

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METABOLIC STUDIES WITH ADRENOCORTICOTROPIN IN CUSHING'S SYNDROME AND IN VIRILISM

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THIS report deals with metabolic studies on a patient with Cushing's syndrome and on one with virilism, following the administration of adrenocorticotropic hormone.

Lewis and Wilkins (1) in a somewhat similar study reported the effects of the administration of adrenocorticotropin over a 24-hour period in 1 patient with Cushing's syndrome and in 2 instances of congenital adrenal hyperplasia with virilism: all 3 patients showed an increase in the urinary excretion of 17-ketosteroids. The patient with Cushing's syndrome showed also an increase in the urinary excretion of the neutral reducing lipids, a rise in the fasting blood sugar level, a fall in the respiratory quotient, a polymorphonuclear leukocytosis, an increase in the excretion of potassium and a marked reduction in the excretion of sodium. The 2 patients with congenital adrenal hyperplasia and virilism, on the other hand, showed only a slight rise in the urinary excretion of the neutral reducing lipids, no increase in the fasting blood sugar level and only a slight fall in the respiratory quotient. Particularly striking, in contrast to the findings in the case of Cushing's syndrome, was the marked increase rather than decrease in the urinary excretion of sodium.

Our report, in addition to the usual steroid, blood sugar, and electrolyte studies, concerns itself particularly with the effect of adrenocorticotropin on calcium, phosphorus, and nitrogen balance.

METHOD

Two patients were selected for study. One was a classic instance of Cushing's syndrome associated with bilateral adrenal cortical hyperplasia, demonstrated by perirenal insufflation. The second case was one of masculinization associated with diffuse luteinization of the ovaries. The patients were studied on the Metabolism Ward of the Hospital, and the duration of

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the metabolic investigation extended over a fifteen-day period in both instances.

In the case of Cushing's syndrome, the period of balance study was divided into five three-day periods. The first two of these three-day periods served as controls and were followed by a three-day period during which the patient received 50 mg. of adrenocorticotropin[1] daily in four divided doses. This was followed by a three-day interval period, with a subsequent three-day period of treatment with testosterone propionate, 50 mg. daily.

The patient with the virilizing syndrome was studied in a similar fashion, except that she had only one three-day control period prior to the administration of adrenocorticotropin, after which she left the hospital for several weeks to return for another three-day control balance period, followed by a three-day period of therapy with testosterone propionate. This report deals only with the effects of adrenocorticotropin.

The patients were given a constant diet, which was prepared beforehand for a 21-day period and divided into 21 portions. The patients were fed this diet for three days before the beginning of the actual control period and it was continued throughout the period of study. The mineral, protein, carbohydrate, and fat constituents of the diet were calculated from standard sources (2, 3, 4). Water was permitted freely. Urine was collected every twenty-four hours, and the stool samples were pooled for three-day periods. The periods were demarcated by distilled water enemas administered before and at the end of each period.

The urinary excretion of the 17-ketosteroids was determined according to

TABLE 1. URINARY EXCRETION OF THE NEUTRAL 17-KETOSTEROIDS (MG./24 HOURS)

	Control period	Period of therapy with adrenocorticotropin
Cushing's syndrome	17.4	37.6
Virilizing syndrome	16.0	26.2

the method of Callow, Callow, and Emmens (5). The 11-oxygenated steroids were determined by the method of Corcoran and Page (6) in which the amount of formaldehyde formed from the oxidation with periodic acid was measured, employing desoxycorticosterone as a standard. Sodium and potassium were determined by means of the flame photometer, calcium by the method of Wang (7), phosphorus by the method of Fiske and Sub-

[1] The adrenocorticotropin was provided by Dr. John R. Mote of the Armour Laboratories, Chicago, Illinois.

barow (8), and nitrogen by the micro-Kjeldahl method. Chloride was measured by the method of Schales and Schales (9).

RESULTS

In the patient with Cushing's syndrome, following the administration of adrenocorticotropin there occurred a considerable increase in the urinary excretion of the 17-ketosteroids, the average 17-ketosteroid excretion during the control period being 17.4 mg. per twenty-four hours, whereas the peak reached during treatment with adrenocorticotropin was 37.6 mg. per twenty-four hours. The urinary excretion of 11-oxygenated steroids follow-

TABLE 2. URINARY EXCRETION OF THE 11-OXYGENATED STEROIDS (MG./24 HOURS)

	Control period	Period of therapy with adrenocorticotropin
Cushing's syndrome	3.43	15.93
Virilizing syndrome	1.14	7.42

ing the administration of adrenocorticotropin rose to approximately four and one-half times the control values, the average during the control period being 3.43 mg. per twenty-four hours, whereas the peak reached during treatment was 15.93 mg. per twenty-four hours. No appreciable change was observed in the blood sugar level during the period of treatment with adrenocorticotropin. Similarly, no glycosuria was observed at any time during the course of study. Following the administration of adrenocorticotropin there occurred a decrease in both the serum sodium and potassium concentration, which was probably an expression of hemodilution. With a daily sodium intake of 81.7 mEq., during the period of administration of adrenocorticotropin, there occurred a marked decrease in the urinary excretion of sodium and a retention of this electrolyte as

TABLE 3. SODIUM BALANCE STUDIES

	Cushing's syndrome		*Virilizing syndrome*	
	Control period	Period of therapy with adreno-corticotropin	Control period	Period of therapy with adreno-corticotropin
Total intake, mEq./24 hrs.	81.7	81.7	81.5	81.5
Total output, mEq./24 hrs.	104.6	30.0	87.8	85.0
Balance, mEq./24 hrs.	−22.9	+51.7	−6.3	−3.5

indicated by the balance study. The average urinary excretion during the control period was 104.6 mEq. per twenty-four hours, whereas during ACTH therapy only 30 mEq. of sodium was excreted per day. With the administration of adrenocorticotropin there occurred no appreciable change in the urinary excretion of potassium. The average 24-hour urinary excretion during the control period was 73 mEq., whereas that during treatment with adrenocorticotropin was 70 mEq. Chloride behaved in the same manner as sodium during treatment with adrenocorticotropin, in that there occurred a marked decrease in the urinary excretion of this electrolyte. Urinary excretion during the control period was 115.6 mEq., whereas during adrenocorticotropin administration it was 48.3 mEq. Although there was no appreciable gain in weight during treatment with adrenocorticotropin, there was a decrease in the urinary volume during therapy. The average 24-hour urinary excretion during the control period

TABLE 4. POTASSIUM BALANCE STUDIES

	Cushing's syndrome		*Virilizing syndrome*	
	Control period	Period of therapy with adreno-corticotropin	Control period	Period of therapy with adreno-corticotropin
Total intake, mEq./24 hrs.	70.5	70.5	70.5	70.5
Total output, mEq./24 hrs.	73.0	70.0	13.4	22.6
Balance, mEq./24 hrs.	−2.5	+0.5	+57.1	+47.9

TABLE 5. CALCIUM BALANCE STUDIES

	Intake, mg./24 hrs.	Output, mg./24 hrs.			Balance, mg./24 hrs.
		Urine	Stool	Total	
Cushing's syndrome					
Control period	773	101.2	758.0	859.2	−86.2
Period of therapy with adrenocorticotropin	773	103.4	2173	2276.4	−1503.4
Virilizing syndrome					
Control period	753	35.5	1067	1102	−349
Period of therapy with adrenocorticotropin	753	29.5	1200	1229	−476

was 848 cc., whereas that during adrenocorticotropin therapy was 710 cc.

The results of the calcium balance studies were interesting. With a constant intake of calcium, which averaged 773 mg. per day during the entire period of study, the total combined daily urine and stool excretion of calcium was 859.2 mg., with a negative balance of 86.2 mg., whereas the combined excretion during treatment with adrenocorticotropin was 2,276.4 mg., a negative calcium balance of 1,503.4 mg. per twenty-four hours. Almost all of the excessive loss of calcium during treatment with adrenocorticotropin occurred in the stool. That this is not only an expression of the failure of absorption from the intestinal tract but in addition must include a marked increase in the fecal excretion of calcium is evident from the fact that the total calcium excretion considerably exceeds the intake.

Phosphorus behaved in a somewhat similar manner to calcium. During the control period, with an intake of 1,126 mg. per day, the average daily combined urinary and fecal excretion of phosphorus was 856 mg., resulting in a positive balance of 270 mg. of phosphours. During treatment with adrenocorticotropin the combined average daily urinary and fecal excretion of phosphorus was 1,200.7 mg., resulting in a daily negative phosphorus balance of 74.7 mg. The effect of adrenocorticotropin in inducing a negative phosphorus balance was more marked within the three-day period after cessation of treatment, at which time there was a daily negative balance of 288.4 mg. of phosphorus.

TABLE 6. PHOSPHORUS BALANCE STUDIES

	Intake, mg./24 hrs.	Output, mg./24 hrs.			Balance, mg./24 hrs.
		Urine	Stool	Total	
Cushing's syndrome					
Control period	1126	578.9	277.1	856	+270
Period of therapy with adrenocorticotropin	1126	720.6	480.1	1200.7	−74.7
Virilizing syndrome					
Control period	1040	554.6	402.8	957	+83
Period of therapy with adrenocorticotropin	1040	541.4	611.6	1153	−113

The administration of adrenocorticotropin resulted in an increase in the total daily excretion of nitrogen. During the control period, the negative nitrogen balance with a daily intake of 11.10 Gm. of nitrogen was 1.12 Gm., whereas with adrenocorticotropin therapy it was 2.85 Gm.

Adrenocorticotropin administration resulted in a leukocytosis, with a reduction in the absolute numbers of lymphocytes and eosinophils. The average total white blood cell count at the peak reached during adrenocorticotropin therapy was 13,200. The absolute number of circulating eosinophils fell from an average of 38 to 0, while the average number of lymphocytes dropped from 2,010 to a minimum of 924. There occurred no appreciable change in either the hemoglobin or the total red blood cell count.

In the second case, the patient with the masculinizing syndrome, there occurred a considerable increase in the urinary excretion of the 17-ketosteroids following the administration of adrenocorticotropin. Whereas during the three-day control period the average daily urinary excretion of the neutral 17-ketosteroids was 16.0 mg. per twenty-four hours, the peak attained during the period of treatment with adrenocorticotropin was 26.2 mg. per twenty-four hours. Similarly there occurred a sixfold increase in the daily urinary excretion of the 11-oxygenated steroids, the average 24-hour control value being 1.14 mg., and the peak being 7.42 mg. during treatment with the hormone.

As in the first instance, there occurred no appreciable change in blood sugar value during the treatment period, as compared to the control, and at no time was glycosuria observed.

There occurred no appreciable change in serum sodium, potassium, or chloride concentration. In contrast to the patient with Cushing's syndrome however, this patient showed no change in the sodium balance following the administration of adrenocorticotropin. With a constant daily sodium intake of 81.5 mEq. during the control period, the average daily excretion of sodium was 87.8 mEq., and during the period of treatment with adrenocotropic hormone was 85.0 mEq. The administration of the hormone caused some increase in the urinary excretion of potassium, although there still remained a strongly positive potassium balance. With a constant daily intake of 70.5 mEq., the average daily urinary excretion of this ion during the control period was 13.4 mEq. and during the period of treatment, 22.6 mEq. It is interesting to note that potassium retention occurred at all times in this patient with virilism. During the control period there was a positive nitrogen balance, but during the administration of adrenocorticotropin there was some loss of nitrogen. On a daily intake of 10.0 Gm. of nitrogen the average daily combined urine and fecal loss during the control period was 8.3 Gm. and during the period of treatment, 11.0 Gm. As with sodium, there occurred no significant change in the daily urinary excretion of chlorides, the average daily urinary excretion during the control period being 99.8 mEq., and during the period of treatment, 94.9 mEq.

During the treatment period there occurred no change in weight and only a very slight decrease in the urinary volume, the daily urinary output

being 1,068 cc. during the control period, and 995 cc. during the period of treatment.

During the administration of adrenocorticotropic hormone there occurred a slight increase in the fecal excretion of calcium in contrast to that which obtained during the control period. On a daily intake of 753 mg. of calcium, during the control period the average daily combined fecal and

TABLE 7. NITROGEN BALANCE STUDIES

	Intake, Gm./24 hrs.	Output, Gm./24 hrs.			Balance, Gm./24 hrs.
		Urine	Stool	Total	
Cushing's syndrome					
Control period	11.10	11.54	0.68	11.22	−1.12
Period of therapy with adrenocorticotropin	11.10	12.81	1.14	13.95	−2.85
Virilizing syndrome					
Control period	10.0	7.16	1.14	8.3	+1.7
Period of therapy with adrenocorticotropin	10.0	9.57	1.40	11.0	−1.0

urinary calcium excretion was 1,102 mg., and during the period of treatment with adrenocorticotropin it was 1,229 mg.

The excretion of phosphorus followed a similar pattern. On a daily intake of 1,040 mg. there was a total daily excretion of 957 mg. during the control period and 1,153 mg. during the period of treatment with adrenocorticotropic hormone.

Although the administration of adrenocorticotropin resulted in some increase in the fecal excretion of both calcium and phosphorus in this patient, it was much less than that observed in the patient with Cushing's syndrome.

There occurred only a very slight increase in the total white cell count following the administration of adrenocorticotropin, and a moderate reduction in eosinophils.

CONCLUSIONS

1. Balance studies during treatment with 50 mg. of adrenocorticotropin daily were made on 2 female patients, 1 with Cushing's syndrome due to bilateral adrenal cortical hyperplasia, and 1 with a masculinizing syndrome associated with diffuse luteinization of the ovaries.

2. In both there occurred a significant increase in the daily urinary excretion of the 17-ketosteroids and the 11-oxygenated steroids. Neither showed an appreciable elevation in the fasting blood sugar level, or glycosuria.

3. In both there occurred a leukocytosis in the peripheral blood and a reduction in the absolute number of eosinophils, although the increase in the total white cell count was less in the patient with the virilizing syndrome than in the patient with the Cushing's syndrome.

4. The patient with Cushing's syndrome showed a marked decrease in the urinary excretion of sodium and chloride, but no change in the urinary excretion of potassium. The patient with the virilizing syndrome, on the other hand, showed no alteration in the urinary excretion of sodium and chloride and a slight potassium diuresis.

5. The most striking change in the patient with Cushing's syndrome occurred in relation to calcium balance. During the administration of adrenocorticotropic hormone, there occurred only a slight increase in the urinary excretion but a marked increase in the fecal excretion of calcium. The fact that the total calcium excretion was considerably in excess of the calcium intake showed that this was not just a reflection of decreased absorption of calcium. Phosphorus behaved in a manner similar to calcium. The patient with virilization, on the other hand, showed only a slight increase in the fecal excretion of calcium and phosphorus after the administration of adrenocorticotropin. In both patients there occurred a slightly negative nitrogen balance during the period of treatment.

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CLINICAL ABSTRACTS

Case 1. Cushing's syndrome

A 25-year-old woman was admitted with a 12-year history of amenorrhea, obesity, abdominal striae, thin skin and easy bruisability, headache, hypertension, and hirsutism.

The physical examination revealed a moonfaced, plethoric woman. The breasts were underdeveloped. The clitoris was enlarged. A mild degree of facial hirsutism was present. There were purplish abdominal striae. The blood pressure was 190 mm. of Hg systolic and 110 mm. diastolic.

The laboratory examination revealed the peripheral blood count and differential white cell count to be essentially normal. The glucose tolerance curve was diabetic in character. There was no glycosuria. The urinary excretion of the neutral 17-ketosteroids was 10.3 mg. per twenty-four hours. The urinary excretion of the 11-oxysteroids was 3.43 mg. per twenty-four hours, which represented a considerable elevation. The basal metabolic rate was minus 16 per cent.

X-ray examination of the spine revealed extensive osteoporosis. In addition, osteoporosis and multiple fractures of the ribs, ischium, and tibia were present. Bilateral perirenal insufflation failed to reveal the presence of a tumor of either adrenal but showed considerable enlargement of both glands. Roentgen studies showed a normal sella turcica.

Case 2. Virilizing syndrome associated with diffuse luteinization of the ovaries

The patient was admitted to the hospital for the first time in 1943, at the age of 20. She had been obese for many years and in the previous three years amenorrhea and hirsutism had developed. Hirsutism of the face was marked and required shaving every other day. In addition, there was considerable hirsutism of the chest, the abdomen, and the extremities.

The physical examination revealed an obese girl weighing 230 pounds, with extensive hirsutism as described. The systolic blood pressure was 120 mm. of Hg and the diastolic 70. There were no purplish abdominal striae. There was considerable acne of the back and chest. The clitoris was enlarged and there was a male pubic escutcheon. Pelvic examination was otherwise normal. The breasts were poorly developed.

The laboratory investigation was as follows: The glucose tolerance curve, employing 1.75 grams of glucose per kilogram of body weight, was entirely normal. The urinary excretion of neutral 17-ketosteroids was 11.4 mg. per twenty-four hours. The concentration of sodium in the blood was 138 mEq./L. X-ray examination of the skull, vertebral column, and long bones revealed no abnormalities. There was no osteoporosis. A bilateral perirenal insufflation failed to show any enlargement or tumors of the adrenal glands.

The patient was treated with irradiation to the pituitary gland without improvement.

In 1946, three years after her first admission to the hospital, she was subjected to a bilateral adrenal exploration. The adrenals were grossly normal in appearance and histologic studies of the biopsy specimens taken from both adrenals showed a somewhat prominent zona reticularis in one gland and no abnormalities in the other.

Subsequently, another exploratory operation was performed. The ovaries and adnexae were visualized and found to be normal in size. Biopsy studies of both ovaries showed diffuse luteinization, and the patient was therefore subjected to a subtotal resection of both ovaries. Following this procedure there was a return of the menses for two months, with no other change in the patient's symptoms or physical appearance. Amenorrhea then recurred and has continued to date.

POSTPARTUM ENDOMETRIAL HYPERPLASIA IN DIABETICS TREATED WITH STILBESTROL AND PROGESTERONE*

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FEMALES with diabetes mellitus are apt to have numerous difficulties associated with pregnancy. For example, the rate of early spontaneous abortion and pre-eclampsia is high, as is the incidence of intrauterine death of the fetus. These and other complications of pregnancy are so common that, according to White (1), a normal obstetrical course in diabetes has been conspicuous by its rarity. Such difficulties occur even with adequate control of the blood sugar.

Hypo-ovarianism in diabetics is also frequent (1, 2) and is associated with such things as amenorrhea, poor follicular development, dwarfism, hypoplasia of the uterus, a low 17-ketosteroid excretion and a high serum level for follicle-stimulating hormone. In the belief that some of the complications of pregnancy are due to a deficiency of ovarian and placental hormones, several hundred diabetics have been treated by Dr. Priscilla White of the Joslin Clinic, with large doses of stilbestrol and progesterone during the course of the pregnancy. An example of a typical course of treatment is as follows:

Week of pregnancy	*Stilbestrol, mg. daily*	*Progesterone, mg. daily*
up to 20th	5	5
20–24	10	10
24–28	15	15
28–32	25	25
32–38	50	50

(The stilbestrol and progesterone were given intramuscularly in oil.)

Clinical results from such treatment have been spectacular. With the corrected hormonal balance, fetal survival was raised from 50 per cent to 90 per cent.

Some patients so treated, with excellent results as far as the course of

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the pregnancy was concerned, had as a complication, postpartum uterine bleeding which clinically appeared to be the result of endometrial hyperplasia. The postpartum bleeding usually began from three to six months, sometimes as long as a year, after delivery, in spite of the fact that no hormonal therapy was given after termination of the pregnancy. There was

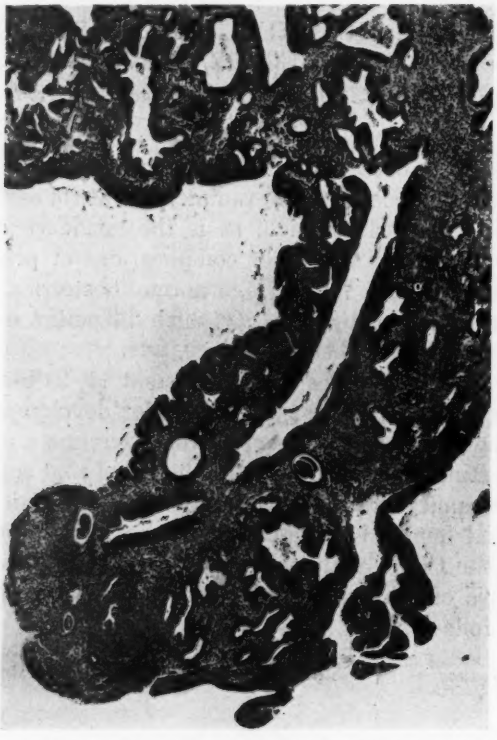

FIG. 1. Cystic and adenomatous polypoid hyperplasia. Eosin-methylene blue. ×50.

never a menstrual period between delivery and onset of bleeding. The hemorrhage was continuous and lasted in some instances for as long as two years. It was occasionally severe enough to require blood transfusions and in every instance a curettage of the endometrium was performed.

This paper is primarily a report of the pathologic study of the endometrium removed from over 50 such patients from three months to two years after delivery.

PATHOLOGY

The pathologic appearance of the endometrium was remarkably uniform and showed no variation with the age of the patient or the severity of the diabetes.

Grossly, the curettings were unusually abundant, often reaching a total volume of 4 to 5 cc. The tissue was pale yellow, soft, moist, shining and relatively friable. It differed from carcinoma curettings, which are ordinarily drier, more granular and friable, and from those of usual endo-

FIG. 2. Adenomatous hyperplasia with stromal overgrowth and marked atypism of glands. Eosin-methylene blue. ×50.

metrial hyperplasia, which as a rule are relatively more edematous and amorphous.

Microscopically, the fragments were frequently polypoid as shown by superficial epithelium covering three sides (Fig. 1). Endometrial glands varied widely in size and shape. Some were typical of cystic hyperplasia, with actively proliferating epithelium; others showed adenomatous outpouchings at single or numerous points. The lining epithelium was frequently quite thick, with marked stratification, some loss of cell polarity and a few papillary projections (Fig. 2).

Some cells had abundant eosinophilic cytoplasm. Cells in mitosis were usually present, but nuclear atypism was not prominent. Many superficial

cells were ciliated. Some fluid and mucus production was often observed. The pattern resembled that of the proliferative phase of the menstrual cycle (Fig. 3). Best's carmine stain showed the presence of focal subnuclear and intraluminal glycogen in some cases; this, however, was the only suggestive evidence of the progestational phase found in any case.

FIG. 3. Stratification of epithelium, ciliated cells and fluid secretion. Note resemblance to proliferative phase. Eosin-methylene blue. ×250.

Proliferation of endometrial stroma was unusually marked at times, with production of broad sheets of close-packed spindle cells which compressed neighboring glands. Mitoses and irregularity in stromal cell size were present as a rule, but no anaplastic alterations were observed. The blood vessels frequently were filled by thrombi, as in ordinary endometrial hyperplasia, but showed no other abnormality. Almost half the cases examined had eosinophilic polymorphonuclear leukocytes abundantly scattered through the stroma (Fig. 4). This is uncommon in either normal or abnormal endometria. A few of the cases showed stromal necrosis, plasma cell infiltration, or phagocytes containing hemosiderin.

The histologic diagnosis in these cases, all of which bore a close resemblance to each other, was endometrial hyperplasia. Polypoid, cystic and adenomatous subtypes were usually found mingled. Chronic endometritis was also considered to be present in 4 cases.

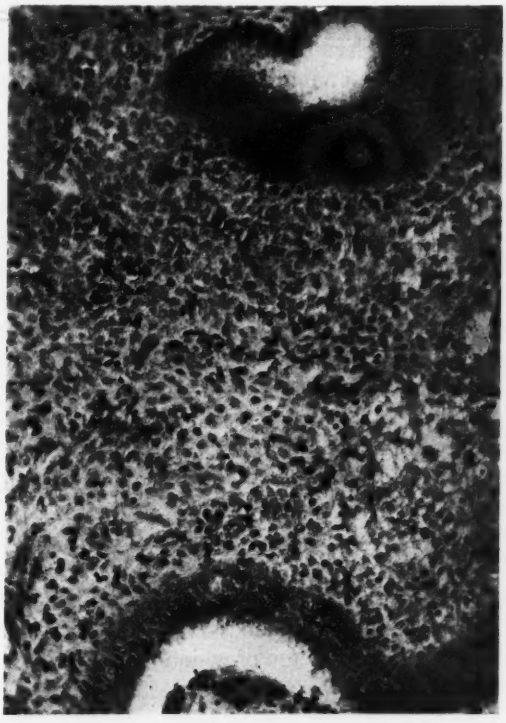

FIG. 4. Stromal overgrowth. Polymorphonuclear cells are eosinophils. Mitoses in epithelium. Eosin-methylene blue. ×250.

COMMENT

One point of interest regarding the pathologic picture of the endometrium in these cases is that it was unlike that seen in various other types of dysfunctional bleeding and was in most instances sufficiently characteristic to allow one to make the diagnosis without aid of history. On superficial examination, it bore a resemblance to the common idiopathic cystic and adenomatous hyperplasias seen in nondiabetic females; it also resembled the hyperplasias of the endometrium which have been produced by estrogens in experimental animals. However, on more detailed examination, it was found to differ from such hyperplasias in that it involved a more regular and florid overgrowth of both glands and stroma, and was quanti-

tatively more marked. The complete picture of alternation of close-packed, tortuous, adenomatous and dilated glands with abundant, compact, cellular stroma often containing eosinophilic leukocytes, was unlike that seen in other hyperplastic states.

The relationship of the hyperplasia to the diabetic state is of interest. In normal women treated with oral stilbestrol during pregnancy, no postpartum endometrial hyperplasias have been noted (3). Only 18 cases of postpartum or postabortal endometrial hyperplasia have occurred in sixteen years (1931–1947) at the Boston Lying-In Hospital and in only 6 of these instances did the hyperplasia resemble that described in the cases of this series. Although adequate control cases are unfortunately not available, it seems possible that the diabetic condition and the accompanying endocrine imbalances did play a role in the development of the hyperplasia. If one subscribes to an idea of anterior pituitary and adrenal cortical overactivity in some diabetics, additional stimuli from slow absorption of estrogen would enhance tissue overgrowth in susceptible sites such as the uterus. In different series of reported endometrial carcinomas, from 9 to 11 per cent are diabetics (4, 5). This rather high incidence suggests a common metabolic disorder in diabetes mellitus, endometrial hyperplasia and endometrial carcinoma.

It is of interest that the postpartum bleeding did not occur in all cases and that the patients who did develop the complication were no different from the others in respect to pregestational menstrual behavior, duration or severity of the diabetes, or age.

It is of further interest to note that although the bleeding occurred uncommonly (less than 5 per cent incidence) in the greater part of the group, there were 50 consecutive cases, each of which developed the complication. The only variable in the treatment of these 50 patients was that they received preparations containing 5 mg. of stilbestrol in 1 cc. of either peanut oil or cottonseed oil; the other patients, who developed the complication uncommonly, received preparations containing 25 mg. of stilbestrol in 1 cc. of either sesame or cottonseed oil. Total dosage of stilbestrol was constant and no trace substances could be implicated; therefore the varying type or varying concentration of the oil vehicle must be suspected as being the cause of the constant appearance of the complication in the 50 consecutive cases. Assays of estrogen in rats have shown similar anomalous results ascribable to differing oil properties (3). Many patients noted a severe inflammatory reaction at the site of oil injection. Experiments in animals indicate that some lipid substances, when injected, may excite an inflammatory reaction with a resultant fibrous capsule formation; such encapsulation would retard absorption. It seems reasonable that certain preparations of stilbestrol had a longer continued action on the endometri-

um because of a slower rate of absorption due to encapsulation. The logical conclusion, therefore, is that the hyperplastic endometrium was often the result not only of large dosages of estrogenic substances in a diabetic, but of prolonged action of the stilbestrol.

One additional point regarding the pathologic appearance of the endometrium is worthy of emphasis. Because of the unusual amount and degree of tissue overgrowth, the first cases of the series were regarded as precancerous. However, further experience and a review of the pathology of the entire group has tempered this opinion. Despite its alarming appearance, the hyperplasia has been self-limited if treated by curettage, and does not prevent subsequent pregnancies.

SUMMARY

The pathologic appearance of hyperplastic endometrium occurring in postpartum diabetics is reported. The diabetic females had been treated during pregnancy with large doses of stilbestrol and progesterone. The hyperplasia was considered to be the result of prolonged estrogenic stimulation in a diabetic. It was not thought to be either precancerous or of serious significance in the light of the excellent clinical results obtained by the therapy. However, the possibility of a common endocrinologic factor in diabetes mellitus, endometrial hyperplasia and endometrial carcinoma is discussed.

Acknowledgment

Acknowledgment is made to Dr. Arthur T. Hertig of the Boston Lying-In Hospital and Dr. P. M. LeCompte of the Faulkner Hospital for some of the material presented in this paper.

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A CASE OF OVARIAN AGENESIS WITH NORMAL URINARY GONADOTROPIN TITER

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THE syndrome of ovarian agenesis has been rather extensively characterized by several authors since Turner's clinical description in 1938 (1–6).

We wish to present an unusual case of ovarian agenesis in which we were obliged to confirm the diagnosis by biopsy of the rudimentary ovarian anlagen because of a consistently normal urinary gonadotropic hormone assay and normal development of the breasts and external genitalia.

CASE REPORT

Mrs. T. A., a 35-year-old Greek female, was first seen in the clinic on June 10, 1949, with a chief complaint of sterility after nine years of marriage. She stated that she had never spontaneously menstruated in her life. Her early developmental history was entirely normal and she had had no major illnesses, injuries or operations. She was first noted to be unusually short when she ceased growing at about 14 years of age. Review by systems revealed no abnormalities. One brother and one sister were well and both had children. Her mother had had a large goiter; otherwise the family history was negative.

This patient's breasts and pubic and axillary hair had developed rapidly when she was about 12 years of age, and normal libido appeared at about that time. She had had normal marital relations with frequent orgasm during the nine years of marriage and had practiced no contraception.

Her sole therapy prior to the present observations had been a course of hormonal injections given intramuscularly at approximately weekly intervals over a period of eight months beginning in November 1946. It was not possible to ascertain the exact nature of the injected material, but it is presumed to have been some form of estrogenic hormone. She had experienced menstrual bleeding for periods of three to five days on four occasions during the course of this therapy. These menses were accompanied by severe cramps and the passage of numerous large clots. In the two years since the completion of this therapy she had had no menstrual bleeding. Her breasts, which had been previously fully developed, showed only a slight increase in turgidity during the course of therapy. According to both the patient and her husband the breasts had been altered in size only negligibly during the course of injections. She had not received any form of medication during the two years preceding the present observations.

Physical examination

The patient's height was 4 ft. 7 ins. and her weight was 109 lbs. She was of stocky build with abundant subcutaneous fat resulting in a feminine contour (Fig. 1). The

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blood pressure was 100/70 mm.Hg. Her skin was clear of eruption, pigmentation or hirsutism. Head hair, axillary hair and pubic hair were all normal in amount and quality. The extremities were short but otherwise normal, with no evidence of webbing of the digits. Her neck was short and thick but no webbing was apparent and the thyroid was not remarkable. Heart and lungs were normal. Breasts were well developed with normal nipples and areolae. The abdomen was rounded and thick-walled. The external genitalia

FIG. 1. Mrs. T. A., 35 years old. Note rapid postoperative growth of pubic hair.

were normally developed (Fig. 2) and introitus readily admitted two examining fingers. The vaginal mucosa was pink and moist. A normal appearing cervix about 2 cm. in diameter was visualized and the os readily admitted a probe to a depth of 6 centimeters. A uterine fundus about 3 cm. in width was clearly palpated, but no adnexal structures were identified. Visual fields and fundi were normal and complete neurologic examination revealed no abnormalities.

Laboratory data

Blood: hemoglobin 13.4 Gm. (89 per cent); hematocrit 44 per cent; W.B.C. 8,100; neutrophils 57 per cent and lymphocytes 43 per cent. Urine: acid, yellow, and turbid, containing no sugar or albumin. Mazinni and Kahn tests gave negative results.

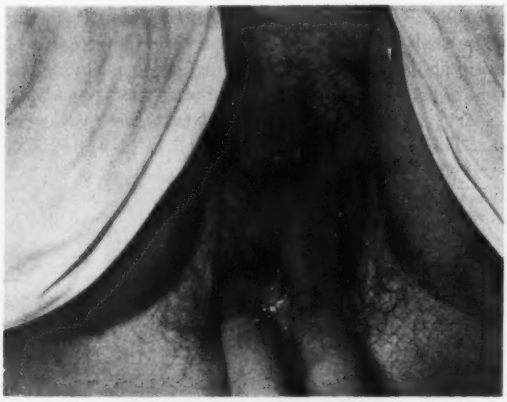

FIG. 2. External genitalia; normal labia, clitoris and vaginal mucosa.

X-ray examination revealed a normal sella and skull. X-ray examination of the long bones and the dorso-lumbar spine showed no abnormalities.

Eight separate urinary gonadotropin determinations made by a modification of the method of Klinefelter *et al.* (7) yielded the following values expressed in mouse uterine weight units: −33; +36; −72; −13; −28; −20; −20; −20; −11. Urinary 17-ketosteroids determined by the method of Holtorff and Koch (8) and expressed in milligrams per twenty-four hours were: 9.5; 5.0; 7.0; 2.0; 8.0; 4.5; and 9.0. Urinary estroids as determined fluorometrically by the method of Jailer *et al.* (9) following the extraction by

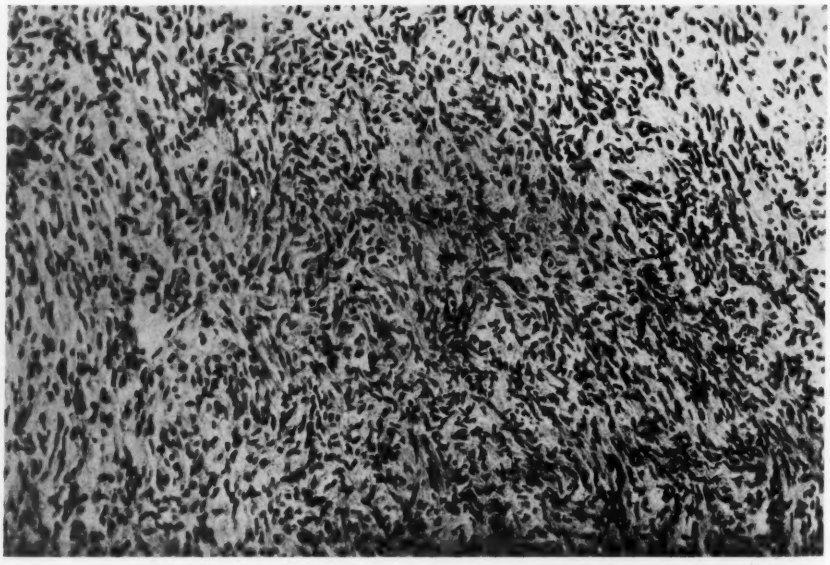

FIG. 3. Histologic section from biopsy specimen of ovarian anlage. ×325.

the procedure of Friedgood *et al.* (10) and expressed in microgram equivalents of estrone per twenty-four hours were: 300, 200, 1,900, 300, 400, 200, and 550.

In view of the difficulty in coming to a definitive clinical diagnosis, an exploratory laparotomy was performed. A careful pelvic survey revealed a complete absence of grossly recognizable ovarian tissue. The ovarian sites were occupied by two strands of nonvascular greyish fibrous tissue about 2 cm. long by 0.5 cm. wide by 0.2 cm. thick. The Fallopian tubes and uterine fundus were normally formed and well vascularized, but of juvenile dimensions. The adnexal regions and parametria were carefully searched for cysts or masses and none was found. A biopsy specimen was taken from each ovarian anlagen. Recovery was rapid and uneventful. The biopsy specimens were fixed in 10 per cent formalin and prepared in a routine manner for staining by hematoxylin and eosin.

Histologic study of the biopsy specimens revealed a dense homogeneous mass of connective tissue (Fig. 3). The cells were typical, elongated fibroblasts and there was very little intercellular matrix. No hyalinization was observed. There was no indication of the presence of interstitial or germinal elements. The histologic picture was quite comparable to that seen in the biopsy material described by Wilkins and Fleischmann (4).

DISCUSSION

This patient is distinctive in that she represents an instance of a failure of the rise in urinary gonadotropin which is characteristic of the syndrome of ovarian agenesis. Moreover, histologic proof of the diagnosis of ovarian agenesis has been previously limited to patients showing elevated gonadotropins. Dorff and associates (11), however, have described a patient presenting many of the features of ovarian agenesis but with no apparent elevation in urinary gonadotropin. No histologic evidence supporting the clinical diagnosis was presented.

The development of apparently normal anatomic and functional secondary sex characters, particularly the breasts and external genitalia, in the total absence of ovarian tissue is especially notable. It is clear from the history that this development of the secondary sex characters was little affected by the course of estrogen therapy. Moreover, two years had intervened between the estrogen therapy and our clinical and laboratory studies.

SUMMARY

A case of ovarian agenesis is presented in which the diagnosis is proved by histologic examination of biopsy specimens of both rudimentary ovarian anlagen and in which lack of elevation of urinary gonadotropin is accompanied by normal development of the breasts and external genitalia.

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CYCLIC CHANGES IN THE VAGINAL BUT NOT IN THE UTERINE MUCOSA OF AMENORRHEIC WOMEN, INDUCED BY A SINGLE INJECTION OF ESTRONE AND PROGESTERONE PRECIPITATES

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THE well known papers of Papanicolaou (1), and Papanicolaou and Shorr (2) demonstrated that the human vaginal mucosa, stimulated by the gonadal hormones, undergoes cyclic changes which are demonstrable in the vaginal smear. The estradiol-induced proliferative phase and the estradiol-progesterone-induced progestational phase of the cycle manifest themselves by characteristic modifications in the vaginal smear, which may, therefore, be used to establish the functional status of the ovary. The vaginal smear technique today represents one of the important routine procedures of gynecologic diagnosis.

We have raised the question whether the information provided by the vaginal smear on the functional status of the uterine mucosa is always reliable and whether the substitution of the vaginal smear method for the histologic examination of the uterine mucosa may be justified.

The present observations were made on amenorrheic women. When a woman, castrated or suffering from longstanding amenorrhea, receives by injection a total dose of 30 to 40 mg. of estradiol benzoate in twenty days, followed by 40 mg. of progesterone for five days, bleeding from a progestational mucosa occurs after an interval of a few days. The study of vaginal smears taken at frequent intervals during the above treatment reveals the characteristic changes of both phases of the normal cycle, proliferative and progestational. The assumption that progesterone is able only to produce bleeding of the uterine mucosa, provided that estrogenic hormone has previously brought about perfect proliferation of the mucous membrane, was shown to be incorrect by Zondek and Rozin (3, 4), who made it clear that it is possible to induce bleeding during the intermenstrual stage (intracyclic bleeding) by injecting 50 mg. of progesterone in the postmenstrual stage, following which bleeding takes place from an im-

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perfectly proliferated mucous membrane containing few glands and no glycogen. These observations led to a simplified treatment of amenorrhea, proposed by Zondek in 1942 (5). It was found that it required only two to five days of treatment with a total dose of 5 mg. of estrone and 25 to 50 mg. of progesterone to induce bleeding in amenorrheic women after an interval of two to three days. Recently Zondek and Rozin (6) demonstrated that the treatment of amenorrhea may be further simplified and that a single injection may induce bleeding provided that the hormones be injected, not in oil solution, but as microcrystalline precipitates.[1] The optimum dose was 5 mg. of estrone and 50 mg. of progesterone. Whereas in the two to five days' treatment with hormones dissolved in oil, bleeding occurred after an interval of only sixty to seventy-two hours, the interval might last for ten to twelve days after the injection of precipitates. Obviously during so short an interval as sixty to seventy-two hours no conspicuous cytologic changes were to be expected in the vaginal smear, at any rate not a biphasic cycle with an estrogenic and a progestational phase. During an interval of nine to twelve days' duration, however, we considered it highly likely that such progressive changes might be demonstrable. Observations showed this to be the case. After injecting an amenorrheic woman with 5 mg. of estrone and 50 mg. of progesterone precipitates, the first changes in the vaginal smear referable to estrogenic activity appear on the second day. Considerable estrogenic activity is present on the third and fourth days and the peak is reached on the fifth day. On the ninth day a typical premenstrual picture is present.

It should be mentioned that injection of 5 mg. of estrone precipitate alone does not induce bleeding in cases of longstanding amenorrhea nor does it induce biphasic changes in the vaginal smear, referable to estrogen stimulation and withdrawal. Six women, 24 to 32 years old, suffering from amenorrhea of two to six years' duration and showing a constant crowded amenorrheic pattern in the vaginal smears upon repeated examination, were taken as controls and injected with 5 mg. of estrone precipitate intramuscularly. Vaginal smears were taken daily and showed the following changes: The first signs of estrogenic activity were present on the second day after injection; increased shedding of basophilic elements and cytolysis were observed. On the third day acidophilic cornified elements appeared and their number increased reaching a peak on the fifth day. The smear became clean on the fourth day. Complete cornification was not observed. The number of acidophilic elements with pyknotic nuclei decreased slowly,

[1] Estrone and progesterone are dissolved in an organic solvent miscible with water, a mixture of various homologous ester derivatives of α-β dichlorhydrin, and the hormones are precipitated from this solution in the syringe by the addition of saline solution (6) (Ambo-Pellettoids Teva, Jerusalem).

with no evidence of folding or clumping. A number of cornified elements were still present on the twelfth to the fourteenth day, the typical crowded smear appearing again on the thirteenth to fifteenth day. No biphasic changes, referable to estrogenic stimulation and withdrawal, were observed in any one of the 6 patients.

We consider the following observation to be important: While all the typical phases of a normal cycle (postmenstrual, ovulative, and premenstrual) are reproduced in the vaginal mucosa during a nine-day period by the action of 5 mg. of estrone and 50 mg. of progesterone precipitates, as shown by the vaginal smear method, no changes or only irrelevant ones are concomitantly induced in the uterine mucosa. Before treatment and at the onset of bleeding, we find a thin uterine mucosa containing few glands, in a state of proliferation and without glycogen, corresponding to a postmenstrual pattern. This supports the view that the vaginal mucosa is much more sensitive to the sexual hormones than is the endometrium. While the vaginal mucosa reacts to 5 mg. of estrone and 50 mg. of progesterone with the typical cytologic changes of the sexual cycle in the course of ten days, the endometrium shows only negligible changes. This fact should be considered before making a diagnosis on the basis of vaginal smears. When we are able to follow the characteristic changes of a luteal phase in vaginal smears, we may infer that progesterone is produced in the ovary but we cannot assume with certainty that the endometrium is in its progestational phase. It appears as if smaller doses of hormones are necessary to induce functional changes in the vaginal mucosa than in the endometrium. In sterile women it is of the utmost importance to establish whether the uterine mucosa is prepared for the embedding of the fertilized egg *i.e.*, if it is in a progestational phase with its glands secreting mucus and glycogen. It is important to determine whether a sterile woman has ovulatory or anovulatory bleeding. In such cases the vaginal smear technique may sometimes lead us to diagnose a progestational phase, while in effect the uterine mucosa is not in a corresponding functional state. Our observations suggest a discrepancy of response between vaginal and uterine mucosa. Consequently the anatomic examination of the uterine mucosa is not to be neglected in favor of the vaginal smear. This conclusion in no way diminishes the importance of the vaginal smear but merely draws attention to the fact that the vaginal secretion is not always a mirror of the functional state of the uterine mucosa.

In another paper we shall show that under certain conditions, for instance in avitaminoses, the opposite may take place, *i.e.*, the uterine mucosa may undergo typical functional changes, while the vaginal mucosa fails to react to hormonal stimuli.

As previously reported (6), 34 patients with amenorrhea were treated

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TABLE 1

Case	Age (yrs.)	Duration of amenorrhea (yrs.)	Findings before treatment		Treatment: estrogen-progesterone (mg.)	Duration of interval (days)	Vaginal smear during interval	Endometrium at the beginning of bleeding (biopsy)
			Vaginal smear	Endometrium (biopsy)				
1	26	2	Crowded, amenorrheic	Early postmenstrual	5–50	10	Biphasic	Early postmenstrual
2	32	5	Crowded	Early postmenstrual	5–50	10	Biphasic	Early postmenstrual
3	31	3	Heavy fluor.	Early postmenstrual	5–50	10	Fluor.	No mucosa obtained
4	25	4	Atrophic	Atrophic	5–50	9	Biphasic, no peak	No mucosa obtained
5	27	2	Crowded, amorrheic	Early postmenstrual	5–50	8	Biphasic	Postmenstrual
6	33	6	Crowded	Early postmenstrual	5–50	10	Biphasic, no peak	Postmenstrual
7	35	9	Atrophic	No mucosa obtained	5–50	No bleeding	Biphasic, no peak	No mucosa obtained (11th day after treatment)
8	28	5	Slightly estrogenic	Postmenstrual	5–50	9	Biphasic	Postmenstrual
9	30	2	Crowded, amenorrheic	No mucosa obtained	5–50	10	Biphasic	Early postmenstrual
10	23	2	Crowded, amenorrheic	Early postmenstrual	5–50	9	Biphasic	Early postmenstrual
11	35	5	Heavy fluor.	Early postmenstrual	5–50	10	Fluor.	No mucosa obtained
12	28	3	Slightly estrogenic	Early postmenstrual	5–50	8	Biphasic	Postmenstrual
13	34	9	Crowded, amenorrheic	Early postmenstrual	5–50	9	Biphasic	Postmenstrual
14	23	4	Crowded, amenorrheic	Early postmenstrual	5–50	9	Biphasic	Postmenstrual
15	30	6	Atrophic	No mucosa obtained	5–50		Biphasic, no peak	
16	27	3	Crowded, amenorrheic	Early postmenstrual	5–50	8	Biphasic, no peak	Postmenstrual
17	29	9	Crowded, amenorrheic	Early postmenstrual	5–50	9	Biphasic	Postmenstrual
18	35	4	Crowded, amenorrheic	No mucosa obtained	5–50	No bleeding	Biphasic, no peak	No mucosa obtained (11th day after treatment)
19	30	4	Heavy fluor.	Postmenstrual	5–50	9	Biphasic	Postmenstrual

with a single injection of estrone-progesterone precipitates. In the meantime, a larger series has been treated in the same way. Altogether the vaginal cytology was thoroughly investigated in 19 of these patients. Three types of reaction were observed. In 3 cases of secondary amenorrhea with considerable vaginal discharge, no evaluation of the changes brought about by hormonal action could be obtained owing to an inflammatory condition of the vaginal mucosa. In 7 cases the vaginal smear did not reach complete cornification, nevertheless a biphasic cycle was quite evident. In 9 cases we observed in quick succession all the phases of a normal cycle. Table 1 tabulates the pertinent data of our observations.

In the following paragraphs is a detailed description of the treatment with estrone-progesterone precipitate in cases of amenorrhea with a "crowded amenorrheic pattern" together with the changes in the vaginal smear before treatment and the typical biphasic changes after the treatment.

Method

The vaginal smear is examined daily for a few days in order to exclude the occurrence of spontaneous cyclic changes. If no estrogenic action is detected and there is a constant "crowded amenorrheic pattern" (basophilic, thin, folded cells of the intermediate type in big clumps and a moderate number of leucocytes), the patient is given 5 to 10 mg. of estrone and 50 mg. of progesterone precipitate in a single injection. Vaginal smears are then examined daily until bleeding starts.

Typical case report

Case No. 37: S. Z. This woman was 29 years old. Menarche occurred at 14. She had had regular menses every twenty-eight days, until nine years previously. She had been married for ten years but had had no pregnancies. The general examination revealed nothing abnormal. The B.M.R. varied from +1 to −4 per cent on repeated examination. A tendency to obesity was easily controlled by diet. Dried thyroid in small quantities

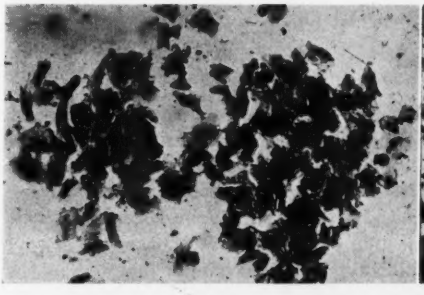

FIG. 1. Vaginal smear before treatment. ×100.

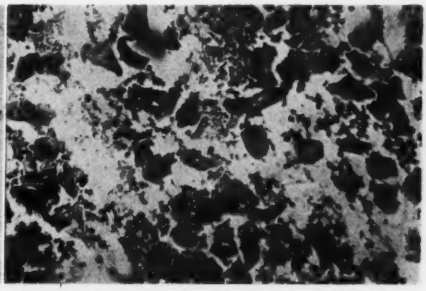

FIG. 2. Second day. ×100.

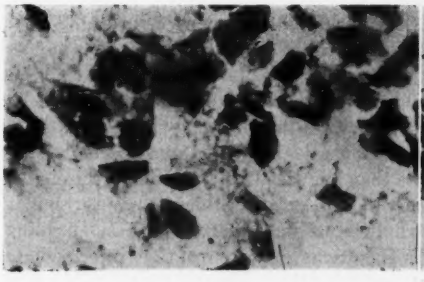

FIG. 3. Fourth day. ×100.

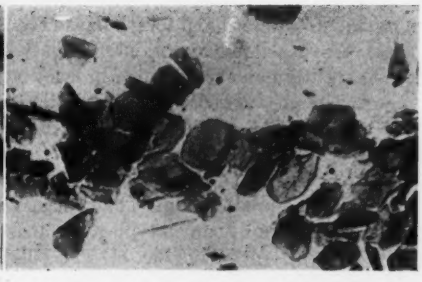

FIG. 4. Fifth day. ×150.

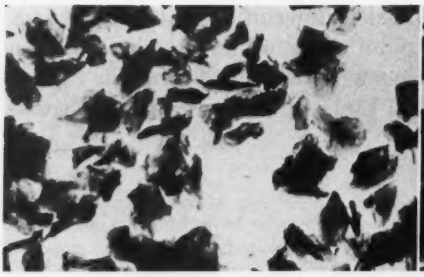

FIG. 5. Seventh day. ×150.

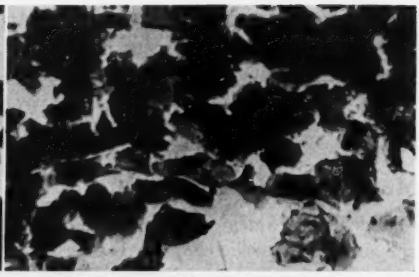

FIG. 6. Eighth day. ×150.

FIG. 7. Ninth day. ×150.

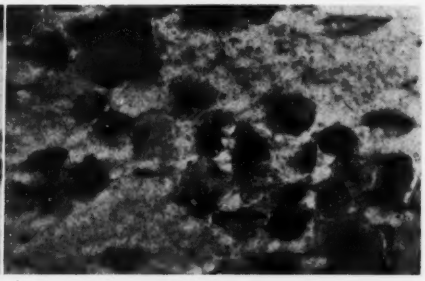

FIG. 8. Tenth day. ×150.

was not well tolerated. Many hormonal treatments in the course of the previous eight years had been unsuccessful. The hypophysis and ovaries were irradiated twice with no results. Repeated biopsies showed a typical thin postmenstrual uterine mucosa. The excretion of FSH in the urine was increased to 100 M.U. per liter. The vaginal smears, repeatedly examined before treatment, showed a constant "crowded amenorrheic pattern" (Fig. 1). On March 7, 1947, an injection of 5 mg. of estrone and 50 mg. of progesterone precipitates was given intragluteally. Striking changes followed in the vaginal smears. The first signs of activity were observed as early as the second day: proliferative activity in the mucosa led to abundant shedding of intermediate basophilic cells. Cytolytic activity was present. The smears were very "dirty" and rich in leucocytes (Fig. 2). The picture changed quickly, considerable estrogenic activity being observed on the third and fourth days (Fig. 3). A fair number of superficial cornified elements were scattered among intermediate basophilic cells, while the smear became increasingly clean and the number of leucocytes decreased. The peak of estrogenic action was reached on the fifth day (Fig. 4) when only large, brightly colored acidophilic cells with clear cytoplasm and pyknotic nuclei were found. On the following two days curling, folding and clumping of acidophilic cornified cells marked the beginning of regression (Fig. 5). The proportion of basophilic elements then increased quickly and by the eighth day they were predominant. The contours of the cells became ill-defined and irregular (Fig. 6). On the ninth day a typical premenstrual picture was observed (Fig. 7). Bleeding started on the tenth day (Fig. 8). The cells in the discharge were from the intermediate and superficial layers and a considerable proportion of them were acidophilic. The flow lasted three days.

The changes described in this case may be summarized as follows: The first five days represented a "follicular phase," which culminated in an "ovulative" smear on the fifth day. A "luteal" phase followed, with rapidly changing patterns from an "early luteal" to a "premenstrual" smear. All the phases of a normal cycle were thus reproduced, although in more rapid succession.

The uterine mucosa was examined before treatment and at the beginning of flow. The changes in the thin, postmenstrual mucosa induced by treatment were negligible. There were only small glands with no signs of function, a picture similar to that in the early postmenstrual phase (Fig. 9). The treatment had a negligible influence on the anatomic structure of the endometrium (Fig. 10).

FIG. 9. Endometrium before treatment. ×40.

FIG. 10. Endometrium at start of bleeding. ×40.

SUMMARY

1. Following a single injection of 5 mg. of estrone and 50 mg. of progesterone precipitates (microcrystals) in amenorrheic women, bleeding is induced after an interval of ten to twelve days. Such bleeding originates in a thin, undeveloped endometrium in a state of proliferation. Daily vaginal smears reveal in many cases a typical functional cycle lasting ten days, as compared with the physiologic period of twenty-eight days. Typical estrogen-induced changes appear in the first five days, and those induced by estrogen-progesterone occur during the next four days.

2. From the fact that the vaginal mucosa (as proved by the vaginal smear changes) reacts to the ovarian hormones, whereas the endometrium at the same time remains unchanged, the conclusion may be drawn that the vaginal mucosa is more sensitive to the ovarian hormones than is the endometrium.

3. The vaginal smear is not always a mirror of the functional state of the uterine mucosa, as was proved in 9 cases of amenorrhea treated with estrone-progesterone precipitates. The presence of progestational changes

in the vaginal secretion shows that the ovary provides progesterone, but it does not always follow that progestational changes take place in the uterine mucosa. In consequence, microscopic examination of the uterine mucosa in order to establish its functional state should not be neglected.

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NEUROTOXICITY DUE TO THIOURACIL AND THIOUREA DERIVATIVES

A CASE REPORT

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THE untoward clinical reactions most frequently noted with the administration of thiouracil and thiourea derivatives have been leukopenia, dermatoses, drug fever and agranulocytosis. Other less common, less publicized, but no less important toxic reactions also occur. These include transient painful swellings of the subcutaneous tissues (1), hematuria (2), edematoid swellings of the face, hands and feet (3–6), spontaneous purpura (7), and acute atrophy of the liver (8). In addition, reactions characterized by a variety of neurologic disturbances may be encountered. These are believed to be of a neurotoxic nature.

It is with these "neurotoxic" reactions that this paper is primarily concerned. An unusual instance of bizarre but definite neuropathy due to 6-n-propylthiouracil (hereinafter called propylthiouracil) focussed our attention upon the heretofore unappreciated importance of the neurologic manifestations of thiouracil and thiourea toxicity.

CASE REPORT

Mrs. M. E. F. (B. G. H. #189509), aged 64, was admitted to the Buffalo General Hospital on March 20, 1947. Twelve months previously she noted the development of nervousness, easy fatigability and heat intolerance. During the three months prior to admission she had lost 100 pounds in weight despite an increase in her appetite. She had also noted dyspnea on exertion. On physical examination this tense, anxious patient showed evidence of marked weight loss. There was a moist, warm skin of fine texture, and a fine tremor of the outstretched fingers. The pulse rate was 120 and the blood pressure 130/60. Bilateral lid lag, tremors of the upper eyelids, widened palpebral fissures and bilateral exophthalmos were present. The eye measurements with the Zeiss exophthalmometer were o.d. 21.0 mm., o.s. 23.0 mm. The thyroid gland was diffusely enlarged, the right lobe being larger than the left. It was firm in consistency; no bruits were heard over the surface. There were fine moist rales at the bases of both lungs posteriorly. The heart was enlarged to percussion and there was pitting edema (1 plus)

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of the ankles. The two-meter chest roentgenogram revealed a cardiothoracic ratio of 0.6 and vascular engorgement in the hilar areas extending into both pulmonary fields. An electrocardiogram showed sinus tachycardia. The serologic findings were normal. The serum cholesterol was 125 mg. per 100 cc. The basal metabolic rate on March 27 (weight 55.4 Kg. and height 148.8 cm.) was plus 90 per cent and on March 28 was plus 83 per cent. On March 28 the administration of 0.400 Gm. thiouracil daily in divided doses was started. There was prompt subjective improvement and by April 21, thirty-five days later, the patient weighed 59.0 Kg. (a gain of 3.6 Kg.) and the B.M.R. was plus 60 per cent. Aside from a mild transient pruritus there were no evidences of thiouracil toxicity. At the time of discharge from the hospital on April 28, 1947, she weighed 65 Kg. and the B.M.R. was plus 55 per cent. The discharge diagnoses were hyperthyroidism and arteriosclerotic heart disease with mild decompensation. The medications to be continued after discharge were thiouracil 0.600 Gm. daily and digitalis 0.1 Gm. daily.

The patient when first seen in the Metabolic Clinic on May 15, 1947, showed a further weight gain, less nervousness, less weakness, and less tremor. Eye measurements with the Zeiss exophthalmometer were o.d. 21 mm. and o.s. 22 mm. Because propylthiouracil had become available, thiouracil was discontinued and a dose of 0.200 Gm. of propylthiouracil daily was prescribed. Clinical improvement continued and after two months of clinic care the B.M.R. was plus 42 per cent, but the cardiac decompensation had become more marked. She was considered a poor surgical risk for thyroidectomy, and the propylthiouracil was increased on August 1 to 0.300 Gm. daily. An even larger dosage was desired, but an inadequate supply of the drug made this impossible. On November 19 her weight was 73 Kg. and her B.M.R. was plus 28 per cent. On December 11, 1947, she complained that two weeks previously she had first noted the gradual onset of a marked burning sensation of the throat, palate, tongue and lips. This was associated with perioral tingling, and the tip of the tongue and the lips felt numb. These subjective sensory changes had become constant. There was no tingling elsewhere. There were no muscle palsies, hyperesthesias, loss of taste, pain, or other neurologic manifestations. The blood calcium, glucose, potassium, urea nitrogen levels and hematologic picture were all normal. Because the tingling and burning became more severe and extremely annoying, propylthiouracil was discontinued on December 18. By January 8, 1948, the dysesthesias had practically disappeared, although digitalis administration was continued. After a period of nine weeks without antithyroid medication the B.M.R. increased to plus 52 per cent, and the fine tremor of the hands and tachycardia had returned. On March 4 she was, therefore, given cyclopropylthiouracil, 0.075 Gm. four times daily. By May 13, the B.M.R. decreased to plus 30 per cent, and on July 15 it was plus 14 per cent. Beginning July 15 she noted again a slight tingling of the tongue but the cyclopropylthiouracil was continued and the tingling gradually subsided. On August 5 she revealed that several days previously she had exhausted her supply of cyclopropylthiouracil and on her own initiative had restarted propylthiouracil, 0.200 Gm. daily. Within forty-eight hours, burning of the tongue, tingling of the tip of the tongue, the inner lower lip and the perioral region had reappeared. As this progressively grew worse during the next forty-eight hours, she stopped the propylthiouracil and the dysesthesias again gradually subsided. On August 5 cyclopropylthiouracil was restarted and by November 4, 1948, the B.M.R. was plus 13 per cent. She had had no further complaints.

The dysesthesias and paresthesias in this patient were associated with the administration of propylthiouracil on two separate occasions, and with 6-n-cyclopropylthiouracil on one occasion. In each instance, the fifth

cranial nerve distally appeared to be solely involved. However, the sensory disturbances associated with the administration of cyclopropylthiouracil were less marked and gradually subsided without interrupting its administration.

DISCUSSION

Our knowledge concerning the distribution of administered thiouracil compounds in various tissues is based almost solely upon the studies of Williams *et al.* (9) in animals. These workers noted that high concentrations of thiouracil were reached particularly in the pituitary, adrenal, thyroid, ovary and bone marrow. Neither brain nor spinal cord changes were present but a detailed study of the entire nervous system was not performed. Reveno (10) has reported the results of a study of the tissues of a woman who died suddenly of a cerebellar hemorrhage while she was under continuous treatment (381 days) with thiouracil for a toxic thyroadenoma. Significant changes were noted in the pituitary, thyroid, spleen and the bone marrow. In the anterior pituitary the disappearance of the eosinophilic elements and the increased basophilia observed simulated the changes noted in the anterior pituitary of thiouracil treated animals.

In view of the unusual neurologic disorder presented by our patient a review of the literature was made in order to gather reports of untoward neurotoxic effects related to the administration of certain goitrogenic compounds or their chemical analogues; *viz.*, thiourea, thiouracil, methylthiouracil, and propylthiouracil. Several instances of toxicity with neurologic manifestations were found.

Thiourea.—Noth (11) described an instance of severe cerebral toxicity which began with weakness and dizziness on the fifth day of treatment with thiourea. The following day the patient became irrational, restless, semicomatose, and incontinent. The respirations were shallow and rapid. Thiourea was discontinued but the symptoms progressed and the patient died two days later. The autopsy findings were not described. Simpson (12) reported a case of delusional psychosis due to thiourea administration. Smirk *et al.* (13, 14) observed in animals receiving S-methylisothiourea an increased respiratory rate apparently due to stimulation of the medullary respiratory centers. During the early investigations of goitrogenic compounds Astwood and Hughes (15) observed in rats a paralysis of motor function from the chronic administration of dithiobiuret, a compound consisting of two thiourea groups. The peripheral nerves, myoneural junctions, and muscles showed no visible structural damage microscopically. It is particularly interesting to note that the entire paralytic process was reversible and disappeared a few days after withdrawing the drug. Altschul (16) also working with dithiobiuret, was able to raise the cortical threshold of

treated animals to electrically induced convulsions. He postulated a functional change in the efferent system of the spinal cord as the most probable site of action of this drug.

Thiouracil.—Neurologic manifestations ascribed to thiouracil include: headache (2, 6, 17, 18); vertigo (19); confusion (20); aural and visual hallucinations (20) toxic psychosis (2, 21, 22); restlessness (17); weakness (17); generalized pains (17, 18); numbness and tightness of the fingers (23); and leg pains (24). Haines and Keating (1) in a preliminary report described an unusual disturbance of the central nervous system which developed in 2 patients treated with thiouracil. Somnolence and confusion in association with myoclonic contractions of various muscles, particularly the facial muscles were observed. The myoclonic twitchings persisted for several days after thiouracil had been discontinued. Although central nervous system irritability reappeared on two subsequent attempts to readminister thiouracil, the exact cause and nature of the reaction in these patients is still in doubt, since a similar reaction was produced in one of these patients by administering an inert substance. Experimentally, tremors and convulsions (25) have been produced in rats receiving toxic amounts of thiouracil.

The report of Holtman (26) is pertinent, for he considers the increased susceptibility to poliomyelitis virus shown in rats given thiouracil to be related to their lowered metabolic state. However, the possibility of a specific neurotoxicity being produced by thiouracil and thereby contributing to this increased susceptibility must not be overlooked. Also, Hines and his co-workers (27) observed that thiouracil-induced hypothyroidism delayed the neuromuscular regeneration of the denervated limb of albino rats. Until now, at least, this has also been believed to be due solely to the effects of a reduction in body growth and metabolism.

Methylthiouracil.—Vertigo (28), paresthesias (28), and loss of taste (29), have been reported as reactions to methylthiouracil. Barfred (28) describes the case of a woman with an adenoma of the thyroid gland who after approximately three months of treatment with methylthiouracil (0.800 Gm. daily) began to note pain and difficulty in controlling the movements of her left leg. Examination showed paresis of the quadriceps on the left side and reduced muscular strength in both legs and in the right arm. There was ill-defined dysesthesia over the left thigh but no objective sensory changes corresponding to the distribution of any peripheral cutaneous nerve could be demonstrated. The spinal fluid was normal. One month after the drug was withdrawn the patient began to walk, and still later showed no residual disturbance of gait or other abnormal neurologic signs.

Propylthiouracil.—Headache (30, 31), numbness of the extremities (32), pain in the jaws and the muscles of the face (31), numbness of the forearms

and hands (33), and emotional disturbances (32), have been reported during treatment with propylthiouracil. In 1 of Crile's (32) 3 patients, who complained of numbness of the extremities, the numbness persisted for several months following withdrawal of the drug. McCullagh (33) described numbness of the forearms and hands which disappeared without stopping propylthiouracil. The persistence and progressive severity of the neurologic disturbances in our patient necessitated a discontinuance of the propylthiouracil, which resulted in a gradual disappearance of symptoms.

There are other factors which should be considered in a discussion of neurotoxicity of the type described in this case report. A nutritional neuropathy may develop in any prolonged hypermetabolic state such as hyperthyroidism in which nutritional demands not only deplete tissue reserves but exceed dietary intake. Functional changes of sufficient severity to produce clinical signs and symptoms of neuropathy may precede the development of anatomic lesions. On this basis, a thiamine deficiency might conceivably develop with its attendant neurologic changes. This condition may even occur in persons who otherwise appear to have normal nutrition. Thiamine depletion may contribute to the development of neurotoxicity in a less direct way by predisposing an individual to damage from neurotoxic metals or drugs (thiouracil derivatives?). The treatment of such neuropathies with dietary measures is ineffective without removal of the toxic agent. It is also of interest to speculate as to a more direct way in which thiourea or thiouracil derivatives may produce an aberration in neurologic function. It is a well established fact that thiourea derivatives produce a therapeutic effect by depressing the peroxidase enzyme system of the thyroid gland. Therefore, if one pictures the function of various nerve structures as being related to a series of enzyme mechanisms in which one of the components has been temporarily "blocked" or made unavailable by a drug such as thiourea or thiouracil then *a priori* neurologic disturbances, not necessarily with pathologic evidence, would appear to be entirely possible. The recovery of the paralyzed animals treated with dithiobiuret upon withdrawal of this drug and the recovery of the patient in this report upon discontinuing propylthiouracil lend support to such an hypothesis.

Indiscriminate condemnation of thiouracil and thiourea derivatives is not implied by this report on their so-called "neurotoxic" reactions. Rather should it be considered as an additional caution in the proper use of these valuable drugs. Neurotoxic reactions constitute an almost negligible fraction of the total reactions (1.8 per cent (Bartels (3)) to propylthiouracil.

It must, however, be emphasized that a neurotoxic reaction may be the sole indication of toxicity to thiouracil and thiourea derivatives. Further, these neurologic disturbances may occur with any dosage. They are not necessarily associated with overdosage. They are less frequent, however,

and less likely with smaller dosages. These reactions have been noted from five days to several months after starting the drugs. Neurotoxicity often subsides promptly upon reducing the size of the dose or upon withdrawal of the drug, but may persist for several weeks.

SUMMARY

A patient exhibiting an unusual neurologic complication consisting of partial involvement of the fifth cranial nerve presumably due to propylthiouracil treatment is presented. Complete recovery followed discontinuance of the medication.

The several types of reported neurotoxic reactions to thiouracil and thiourea derivatives are reviewed.

Neurotoxicity may be the first, the sole, and the most pronounced manifestation of intoxication with the thiouracil and thiourea drugs.

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DYSTROPHIA MYOTONICA, WITH SPECIAL REFERENCE TO ENDOCRINE FUNCTION (KLINEFELTER'S SYNDROME)*

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INTRODUCTION

DYSTROPHIA myotonica is an heredo-degenerative disease with protean manifestations. Among the most frequently encountered of these are myotonia, muscle atrophy, cataracts, premature senility and hypofunction of the endocrine glands. The many aspects of the disease have been thoroughly reviewed in recent years by Waring (1, 2, 3, 4).

The nature of the endocrine disturbance has not been clearly established, however, and there are various theories as to the actual site of the pathologic changes. Three cases of dystrophia myotonica, 1 with a necropsy, are here presented, with a discussion of the endocrine features in relation to the existing theories. Two of these cases present also the symptom complex of gynecomastia, aspermatogenesis, high urinary gonadotropins, small testes and low urinary 17-ketosteroid level, described by Klinefelter *et al.* (5).

CASE REPORTS

Case 1: A 56-year-old white male gave a typical history of myotonia and muscle atrophy since the age of 20. He married and apparently had a normal sexual life but during fifteen years of marriage his wife had no pregnancies, although contraceptives were not used. *Physical examination* revealed progeria, muscular atrophy and myotonia, punctate opacities in the lens cortex bilaterally, moderate gynecomastia and small testes. *Laboratory studies* gave results which were within normal limits with the following exceptions:

17-ketosteroids (urinary)[1]	2.8 mg./twenty-four hrs.
gonadotropins (urinary)[1]	96 I.U./twenty-four hrs.
B.M.R.	minus 18 per cent
Glucose tolerance—fasting	77 mg. per cent
1 hour	150 mg. per cent
2 hours	161 mg. per cent

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[1] Gonadotropins were determined by the mouse uterine weight method modified after Fluhmann; and the 17-ketosteroids were determined by the Holtorff-Koch method.

This patient was admitted to the hospital because of severe malnutrition and avitaminosis which were the results of inability to care for himself properly because of myotonia and weakness. In the hospital he had an attack of acute left ventricular failure with auricular flutter. The administration of testosterone propionate, 25 mg. daily, during one period of his treatment seemed to improve his strength and quinine relieved the myotonia. Nine months after admission he died of intercurrent bronchopneumonia.

Necropsy revealed widespread muscular atrophy and atherosclerotic heart disease. The testes were small, atrophic and showed advanced atrophy and hyalinization of the

FIG. 1. *Histologic section from testicular biopsy specimen (Case 1).*

Note complete hyalinization of seminiferous tubules with sclerosis of lamina propria and basement membrane. No germinal or Sertoli cells are present. The Leydig cells appear diminished in number. ×400.

tubules with complete aspermatogenesis (Fig. 1). The Leydig cells were prominent in some areas but poorly developed in others. There was ductal epithelial proliferation of the breasts. The adrenals showed cortical lipoid depletion, congestion and considerable arteriosclerosis. The thyroid was small in size and the pituitary and parathyroids were essentially normal. The pancreas showed mild hyalinization of the islets of Langerhans.

Case 2: A 48-year-old white male, the brother of the preceding patient, had a history of progressive weakness of three months' duration, and entered the hospital with the primary complaint of gangrene of his right fifth toe. He had led a fairly normal life and

was unmarried; there was a history of normal erections and ejaculations. *Physical examination* revealed progeria, absence of body hair, myotonia, typical muscular atrophy with "hatchet-face" and bilateral ptosis of the eyelids. There were opacities in both lenticular cortices. Moderate gynecomastia was present and the testes were small. The results of *laboratory studies* were essentially normal except for a low B.M.R (minus 36 per cent), a urinary 17-ketosteroid level of 3.5 mg./twenty-four hrs. and a Kepler-

FIG. 2. *Histologic section from testicular biopsy specimen (Case 2).*

Note complete hyalinization of seminiferous tubules, absence of germinal and Sertoli cells with sclerosis of basement membrane and tunica propria. The Leydig cells appear normal and are normal to increased in number. ×160.

Power water excretion test "A" value of 0.82. The urinary gonadotropin excretion was normal (14 and 24 I.U./twenty-four hrs. on two occasions). Microscopic examination of *testicular biopsy* specimens showed complete hyalinization of the seminiferous tubules with absence of germinal and Sertoli cells. The Leydig cells appeared normal (Fig. 2).

There was delayed healing of the right fifth toe amputation site. Finally, however, administration of testosterone propionate, 25 mg. intramuscularly daily, seemed to promote healing. His myotonia was relieved by the administration of quinine and he was discharged considerably improved.

Case 3: A 46-year-old colored woman gave a history of progressive weakness over a period of five years and of failing vision since the age of 20. She had never had a menstrual period, and had never married. *Physical examination* revealed progeria, myotonia, muscular atrophy, senile cataracts bilaterally, and a narrow, virginal vagina which barely admitted the smallest speculum. Neither the cervix nor the uterus was palpable. No breast development was evident, and pubic and axillary hair was sparse.

Laboratory studies revealed:

B.M.R.	minus 9 per cent
Serum cholesterol	414 mg. per cent
Urinary 17-ketosteroids	1.2 mg./twenty-four hrs.
Urinary gonadotropins	46 I.U./twenty-four hrs.

DISCUSSION

Glandular involvement: Although the genetic defect which underlies the disease dystrophia myotonica produces muscular disability and cataracts as the most evident and disabling manifestations of the disease, the endocrinopathies which often accompany these other features would appear to be of much importance and to play a considerable role in the symptomatology. There has been too little emphasis, we believe, upon this particular facet of the pathologic picture. The newer methods of study, including bioassay and glandular biopsy, have supplemented the paucity of postmortem studies dealing with the endocrines, and have shown the profundity of functional impairment of some of the endocrine glands.

Though we are accustomed to expect involvement of the pituitary in pluriglandular syndromes, it is of great interest that there has been no good evidence to implicate this gland in dystrophia myotonica, and our findings in the one case in which there was a postmortem examination are in keeping with this observation. There is, however, an obvious need for further study along this line.

There is, likewise, no good evidence to implicate the parathyroids, although some authors have attempted to explain the cataracts on the basis of hypofunction of these glands. The evidence presented by Greenfield is especially conclusive in this respect (6). The necropsy in Case 1 of our series did not present any evidence of abnormality of these glands.

It has been claimed that the thyroid gland is frequently palpable and of firmer consistency than normal in dystrophia myotonica. This was not true in our cases. The basal metabolic rate was low in 2 of the 3 cases, however, and this is in accord with the usual findings reported in the literature (4), metabolic rates in the range found in myxedema being of frequent occurrence. Despite this fact and the oft-noted premature atherosclerosis associated with the disease, the serum cholesterol is often normal (4). This was true of our 2 men (Cases 1 and 2), but in the woman (Case 3) the serum cholesterol was elevated to 414 mg. per cent.

The glucose tolerance curve in dystrophia myotonica is often suggestive of mild diabetes mellitus. In our Case 1 there was evidence of abnormal glucose metabolism and on postmortem examination there was found a mild degree of hyalinization of the islet cells of the pancreas. It is possible, of course, that the disturbance of gluocose metabolism might be much more severe, were it not for the association of adrenal insufficiency. That the latter was present in our cases was suggested by the postmortem findings in Case 1, by the abnormal Kepler-Power test in Case 2, and by the very low 17-ketosteroid excretion in Case 3.

The hypogenitalism of dystrophia myotonica is a very definite part of the clinical picture. In the male, this deficiency includes testicular atrophy, loss of libido, and diminished or absent fertility (4). Changes in the secondary sex characteristics are not common. The mental attitude of the patient is such, however, that he seldom finds reason to complain of his deficient sexual potency. In women the hypogonadism is manifested by atrophy of the ovaries and uterus with amenorrhea.

Relationship to Klinefelter's syndrome: The discovery that hormonal assay and microscopic examination of testicular tissue in our male patients presented findings which are in correspondence with the criteria for the Klinefelter syndrome as enumerated by Heller and Nelson is, we believe, of considerable interest. This syndrome as originally described by Klinefelter *et al.* in 1942 (5), included gynecomastia, aspermatogenesis with aleydigism, increased urinary excretion of gonadotropin, small testes and decreased urinary excretion of 17-ketosteroids, and was thought to be associated with a primary dysfunction of the seminiferous tubules, the function of the Leydig cells being normal or only slightly disturbed.

Heller and Nelson in 1945 (7–9) presented an enlarged concept of the Klinefelter syndrome, embracing cases with aleydigism, hypoleydigism or isoleydigism. All their patients presented three constant features: 1) small testes, 2) azoospermia, and 3) high urinary gonadotropins. The Leydig cells and the urinary 17-ketosteroids varied from subnormal to normal.

In our male patients there was found complete hyalinization of the seminiferous tubules with absence of germinal and Sertoli cells. The testes were small and there was a marked diminution of urinary excretion of 17-ketosteroids. Gynecomastia was also present. The Leydig cells in Case 2 were histologically normal in appearance, whereas those in Case 1 were grossly abnormal.

This picture suggests that the syndrome of hypogonadism with predominant involvement of the seminiferous tubules may be associated with variable features in other respects; and that further study is needed to elucidate

in any given case the significance of the Leydig cell function and gonadotropin levels and whether or not these are related to each other in any manner.

The female patient is of great interest as far as the endocrinopathy is concerned, since it may be fairly assumed that the genetic defect manifesting itself in her is the same as that in the men and that the glandular manifestations must be considered the analogues of those in the male. The primary amenorrhea, the absence of a palpable uterus and ovaries, and the lack of breast development clearly indicate the hypogonadism existing in this patient.

The significance of the gonadotropin levels: Neither the explanation of Klinefelter *et al.* (the tubular production of "Inhibin"), nor the hypothesis of Heller and Nelson (the utilization of FSH by the tubules) can explain the discrepancy in urinary gonadotropin excretion between the two brothers (Cases 1 and 2), since both of them had completely hyalinized tubules.

The Smiths (10) have presented evidence that placental syncytial cells utilize chorionic gonadotropin, in the production of progesterone and estrogens. It might be hypothesized analogously that the Leydig cells utilize pituitary gonadotropin. This would explain the finding of a high gonadotropin level in Case 1 with abnormal Leydig cells and the normal gonadotropin level in Case 2 with normal Leydig cells.

SUMMARY

Dystrophia myotonica is an hereditary disease associated, in its fully developed form, with cataracts, muscle atrophy and myotonia, progeria, and prominent endocrine disturbances. Three cases of this disease from two families are presented, with special reference to the endocrine abnormalities. In the 2 male patients the diagnostic criteria for the so-called Klinefelter syndrome were satisfied. It must be concluded, therefore, that this hypogenital syndrome includes the genetic defect of dystrophia myotonica among its possible etiologic factors. The female patient demonstrated the interesting association of the clinical features of ovarian agenesis with dystrophia myotonica.

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THE LEVEL OF THE CIRCULATING EOSINOPHILS FOLLOWING TRAUMA

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IT HAS been demonstrated that following the stress of injury, there is at first an increase in the daily excretion of the urinary corticoids and a simultaneous decrease in the urinary excretion of the neutral 17-ketosteroids. Although in severe injury these phenomena have been noted to persist (1) (2) for weeks, more recently it has been found that this period may be only of a few days' duration. Subsequent to this initial phase, the urinary corticoids decrease and the urinary neutral 17-ketosteroids increase in quantity, indicating, it is believed, an alteration in body metabolism from the anti-anabolic first phase to the anabolic second phase.

The administration of adrenocorticotropic hormone results in a decrease in the circulating eosinophils (3). A study was undertaken to learn whether the level of the circulating eosinophils might reflect alteration in urinary steroid excretion. Laragh and Almy (4) meanwhile showed that the number of circulating eosinophils falls markedly following operative procedures.

METHODS

Daily eosinophil counts were performed on fasting subjects (3) following surgery, coronary occlusion, and the administration of adrenocorticotropin, 17-hydroxy-11-dehydrocorticosterone, and testosterone (Table 1). Finger blood was employed.

RESULTS

Simple surgical procedures

Nine patients on the gynecologic service were studied before and after simple surgical procedures such as a vaginal plastic repair or a hysterectomy. The number of circulating eosinophils fell markedly within four hours after operation. This low level persisted for one to three days postoperatively and was then followed by a rise to levels above those noted preoperatively. In 1 patient with fever due to infection, the circulating eosinophils were depressed for a period of over a week.

Coronary occlusion

Studies carried out in 5 patients with coronary occlusion revealed a

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TABLE 1

Pt.	Control eosinophil count per cu. mm.	Procedure	Eosinophil count per cu. mm. (No. of days after procedure)																						
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
1	281	Surgery	78	170	214	391	582		338	394	358	370	384													
2	103	Surgery	50	8		98	122	192	222	292	181	133	171	172												
3	159	Surgery	66	216	445	123	216	289	280	261	203	202														
4	63	Surgery	0	2	19	33	33		75	22	47	50	113	58	75	66	83	63	54							
5	48	Surgery		59	70	113	197	170	143	130	105	73	97													
6	347	Surgery	20	45	108	166	97	120	181	204																
7	169	Surgery	6	127	289	348	300	341	448	345	241	281	234													
8	120	Surgery	0	4	23	167	111	211	161	114	107	117														
9	77	Surgery	5	95	189	252	232	188	184	318																
10		Coronary occlusion							341	305	185	219	213	213												
11		Coronary occlusion		3	11	27	61	114	259	302	230	284	244	219	198		105	130	146	144	120	192				
12		Coronary occlusion		80	0	2	33	147	180	105	23	58														
13		Coronary occlusion				30	69	86	109	59	42	25	33	56	31	47										
14		Coronary occlusion	155	177	311	747		441	669	730	604		450			531		480								
15	47	ACTH*	0	0	0	11	33	48	63	95	119															
16	412	ACTH*	325	177	161																					
17	88	ACTH*	19								92	108					213	191								
18		ACTH*	0	0																						
19	87	ACTH*			1				80	45			250		191					136						
20	117	Compound E**	8					28							5								73		21	21
21		Compound E**		1				17					9								0					
22	164	Testosterone†	147	147	167																					
23	102	Testosterone†	101	131	116																					

* ACTH administered ————

** Compound E (Cortisone) administered .....

† Testosterone administered — — — —

pattern similar in type to that noted following surgery. For obvious reasons, no control values were obtained prior to the thrombosis.

Administration of adrenocorticotropic hormone

Five patients were given adrenocorticotropin. While adrenocorticotropic hormone was being administered, the number of the eosinophils in the circulating blood was markedly decreased. However, in 2 instances (hyperthyroidism) the eosinophil count returned to its original level while the administration of 100 mg. daily of adrenocorticotropin was continued.

When adrenocorticotropin was discontinued, the eosinophil count rose markedly.

Administration of 17-hydroxy-11-dehydrocorticosterone (compound E)

Following the administration of Cortisone to two patients, the eosinophil count was markedly reduced.

Administration of testosterone propionate

When testosterone propionate in oil was administered in doses of 50 mg. a day for three days, no significant alteration in eosinophil count was noted in the 2 patients studied.

DISCUSSION

Since the administration of adrenocorticotropin and compound E resulted in a decrease in the number of circulating eosinophils, and an increase is noted in the urinary excretion of corticoids during the first phase of reaction to trauma, it appears likely that the decrease in circulating eosinophils in this period reflects the increased secretion of adrenocorticotropin with a resultant increase in corticosterones. The secondary rise in the peripheral eosinophil level probably indicates diminution in corticoid secretion rather than a rise in excretion of the anabolic fractions, such as the androgens, since the administration of testosterone is without effect on the level of circulating eosinophils.

Although testosterone propionate does not produce an alteration in the eosinophil level when administered for a period of three days, it does produce a decrease in the urinary excretion of corticoids if given for longer periods (2, 5). This action is presumably mediated through the adenohypophysis.

SUMMARY

A pattern of eosinophil response to stress is found following trauma. At first there is a marked fall in the number of circulating eosinophils, followed by a rise to levels above that of the initial basal level. This pattern is apparently related to the level of adrenal corticosterone secretion.

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Endocrine Review

THE PHYSIOLOGIC BASIS FOR CLINICAL APPLICATIONS OF PROGESTERONE

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THE stimulus which led to the isolation of progesterone originated in the death-bed scientific legacy of Gustav Born. L. Fraenkel, his student, showed that rabbit embryos fail to survive when the corpora lutea are removed (1). Allen and Corner found that extracts of the corpus luteum maintained the pregnancies of ovariectomized rabbits (2). These experiments, in general, substantiated Born's hypothesis that the corpus luteum is an organ of internal secretion which is essential for pregnancy in mammals.

The intensive studies of Corner and Allen led to the isolation of progesterone, the hormone of the corpus luteum. This, in turn, prompted experiments designed to determine whether progesterone could duplicate the biologic activity of the corpus luteum of the human as well as of the laboratory mammal. These studies form the basis of the clinical therapeutic applications of progesterone. The clarity and reproducibility of the physiologic observations bear a close relationship to the specificity and efficacy of progesterone therapy.

BIOLOGIC SOURCES

The corpora lutea of sows were the first source of crystalline progesterone (3, 4, 5, 6). It was found later that progesterone was also contained in the ox adrenal gland (7, 8).

The corpus luteum, placenta, and adrenal cortex of humans have been examined for this steroid. Progestational activity has been demonstrated in placental extracts (9, 10, 11). Similar observations have been made with extracts of the corpus luteum and fetal adrenal cortex (12, 12a, 13, 14, 15). In lieu of more direct evidence, these studies have been interpreted to signify that progesterone is secreted by the human placenta and corpus luteum. Since other steroids demonstrate the progestational activity of progesterone and give rise to similar metabolic excretion steroids (preg-

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nanediol), the identity of the progestogens of these glands cannot yet be considered established.

Pregnanediol excretion studies indicate that relatively large quantities of progesterone or progesterone-like steroids are secreted physiologically. Only slight biologic activity has been obtained from relatively large amounts of placental and luteal tissue. This discrepancy suggests that the hormone is secreted almost as rapidly as it is formed.

BIOLOGIC ACTIVITY

The endometria of rabbits mated to sterile bucks demonstrate a characteristic histologic appearance. Bouin and Ancel observed that this pattern is due to the presence of the corpus luteum (16). Corner suggested that the corpus luteum prepares the uterus for gestation by promoting the development of the progestational endometrium (2). Corner and Allen employed this function of the corpus luteum as a bio-assay criterion in their attempts to concentrate the active principle from the gland (17). The crystalline steroid progesterone was isolated during these studies. Its capacity to induce the endometrial changes characteristic of corpus luteum activity led to its identification and acceptance as the corpus luteum hormone.

The cyclic alterations of the human endometrium were described by Hitschmann and Adler (18). Schroeder (19) and R. Meyer (20) correlated the histologic appearance with the changes observed in the ovary. As with rabbits, the development of the progestational endometrium coincides with the formation of an active corpus luteum. At present, one can only approximate the exact time relations between ovulation and the initiation of luteal activity. Similarly, the interval between the onset and cessation of corpus luteum secretion and endometrial response to these functional changes is still an approximation.

Descriptions of the human endometrium indicate that the gradual development of the progestational (secretory, progravid, differentiative) endometrium begins within the first 24-hour postovulative period. Maximal differentiation occurs after seven to ten days and in the absence of fertilization, regressive premenstrual changes occur (21, 22). If fertilization intervenes, the endometrium demonstrates decidual characteristics which resemble those evoked by Loeb when he disturbed the endometrium of guinea pigs in the luteal phase (21, 23).

Early studies indicated that the progravid response could be elicited in castrate animals more consistently and with smaller amounts of the corpus luteum hormone if its administration were preceded by the injection of estrogen (24, 25, 26). Secretory endometrium was developed by progesterone alone in animals castrated just prior to therapy (27) and in animals with intact adrenal glands (28). Since it has been demonstrated that the

adrenal cortex elaborates estrone, one may question whether progesterone, in the absence of endogenous or exogenous estrogen, can develop secretory endometrium (29).

There were attempts made to develop a progravid endometrium in the human before crystalline progesterone was available. Kaufmann's photographs demonstrate that the administration of estrogen and progestin changed the atrophic endometrium of a castrate woman to the secretory phase (30). Elden (31) and Neustadter (32) could not reproduce Kaufmann's results. Ryden in 1947 (33*) and Segaloff in 1948 (34*) reviewed the reports pertinent to development of the progravid endometrium with progesterone. Although the amounts of progesterone and estrogen varied in these studies, it is apparent that secretory endometrium was not observed with any degree of consistency. Bradbury and co-workers studied the effects of these hormones in women who had had normal menstrual cycles prior to castration. Their results indicate that these castrate women have secretory endometria after the administration of estrogen (0.5 mg. estrone per day) and progesterone (50 mg. daily) (35). Guterman and Frank (36) administered similar amounts of these hormones but have failed thus far to demonstrate unequivocal progravid endometrial changes in amenorrheic women who received the hormones by mouth.

Several laboratory observations may in part explain these divergent results. It is possible that insufficient amounts of progesterone were employed in the early studies. Bradbury and co-workers administered larger doses of progesterone than were available in the early thirties (35). Hisaw and Leonard demonstrated that the progravid response evoked by a given quantity of progesterone could be inhibited if relatively large amounts of estrogen were administered prior to the injection of corpus luteum hormone (24). Courrier and Poumeau-Delille pointed out that, in addition to the fact that less than minimal doses of progesterone will not elicit the response, the development of secretory endometrium depends on the ratio between the administered estrogen and progesterone (37). They observed that when the estradiol:progesterone ratio was maintained at 1:1000, it was possible to reproduce the progestational response in rabbits over a wide range of dosage. The endometrium did not demonstrate secretory changes when the amount of estrogen relative to progesterone was increased. Such a ratio has not been established for humans. If it is demonstrated that an optimal estrogen:progesterone ratio is necessary for the development of human secretory endometrium, it should be possible to elicit this response consistently, given adequate amounts of the hormones.

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* The asterisk signifies that the reference includes a review of the phase of the subject under discussion.

The maintenance of the progravid endometrium presents additional problems. It was noted in rabbits that although progesterone administration continued, the progravid endometrium regressed and appeared atrophic (24, 38). Bradbury and co-workers (35) could not maintain human endometria in the secretory state beyond four weeks although the administration of estrogen and progesterone was continuous. Segaloff and co-workers (39) observed atrophic endometria in women who had pellets of progesterone implanted subcutaneously for several months. It is apparent that there are unrecognized factors necessary for maintaining endometria over long periods of time.

The influence of ovarian hormones on the contractions of the nonpregnant human uterus has been warmly debated since Knaus reported his observations on uterine contractions during the various phases of the menstrual cycle (40). Knaus noted that, after the flow, spontaneous rhythmic contractions occur which are accentuated by the administration of pituitrin. Shortly after ovulation, the contractions were minimal and their reaction to pituitrin was slight. In the few days preceding menstruation, the contractions increased in amplitude and responded vigorously to pituitrin. These findings agreed well with in vitro observations on the contractility of strips of rabbit uterine muscle in the preovulative and postovulative state. In addition, the uterine muscle of rabbits treated with progesterone failed to respond to pituitrin. Thus, it was concluded that progesterone inhibits the spontaneous contractions of the uterus as well as its contractile response to pituitrin. Not all investigators have been able to confirm Knaus' findings (41, 42, 43, 44). Reynolds (45*) reviewed the subject and indicated that the failures to confirm Knaus were in part due to technical differences.

There is sharp divergence of opinion on the influence of exogenous progesterone on uterine contractility and uterine response to pituitrin (42, 44, 45, 46). Krohn, Lackner, and Soskin observed that progesterone diminished spontaneous contractions as well as the response to pitruitrin (46). Henry and Browne followed Knaus' technique "as closely as possible" and observed no diminution in contractile response to pituitrin following progesterone therapy (44*). Adair and Haugen (47) studied isolated strips of human uterus in vitro. The muscle strips obtained in the postovulative phase and from patients treated with progesterone, on the whole, showed less response to pituitrin than those not influenced by progesterone.

The in vivo studies were usually carried out by first following kymographic tracings of uterine contractions for a variable period. The hormone was then administered and the observations were continued for several hours. The patients received intramuscular injections of as much as 20 mg. of progesterone in oil solution. Several questions are raised by these

experiments. Was sufficient hormone absorbed in the few hours of the experiment to influence uterine contractions? What effect would the injection of the oily vehicle itself have on the patterns observed? At present, further and more detailed study is indicated to resolve the differences observed by various investigators. In addition to the ovarian and posterior pituitary hormones, there may be factors, heretofore unrecognized, which have a significant function in regulating uterine contractility.

It is clear from Bartelmez's review on menstruation (48*) that progesterone is not essential for either cyclic or noncyclic uterine bleeding. Cyclic bleeding may normally occur in the human in the absence of detectable progesterone secretion and may be imitated by the cyclic administration of estrogen. Progesterone may influence bleeding after the uterus has been stimulated by administered or secreted estrogen (49).

In the normal menstrual cycle, the estrogenic hormones promote the growth of endometrial glands and blood vessels. The glands are straight and demonstrate some secretory activity. Coiling of the arterioles occurs in the deeper portion of the membrane. The added secretion of progesterone leads to further coiling of the arterioles and the corkscrew appearance of the glands which demonstrate increased secretory activity. The endometrium loses fluid upon the cessation of the secretion of these hormones. The decreasing thickness of the membrane leads to further coiling and compression of the arterioles. The flow of blood is diminished and vascular spasm occurs. Edema, necrosis, and the formation of superficial hematomata are followed by the bloody shedding of the endometrium (48*).

The contribution of the coiled arterioles to the bleeding phenomenon has been emphasized by Daron (50) and Markee (51). Kaiser has observed that menstruation and bleeding may occur in some New World monkeys which do not have endometrial coiled arterioles (52). Bleeding can be provoked in other primates and humans upon the cessation of estrogen administration. Yet estrogen does not cause much coiling of the superficial endometrial arterioles. The marked development of the coiled arterioles under the influence of progesterone indicates their extensive capacity for adaptation to pregnancy (53). The changes exhibited by these vessels upon withdrawal of the sustaining hormones represents an exaggeration of the pattern obtainable with estrogen alone. Although the administration of large quantities of progesterone to castrate monkeys has evoked bleeding, the possible influence of estrogenic hormone from the adrenal cortex has not been eliminated (28).

Aside from the observations that steroid hormones can induce bleeding in experimental animals by direct action on the endometrium, there is little to indicate how these substances initiate the changes which result in uterine

bleeding. Smith and Smith have extracted a protein from the endometrium which is associated with shedding of the membrane in the nonpregnant and pregnant states (54, 55, 56). The significance of this "menstrual toxin" which causes spasm of the endometrial arterioles awaits further study.

In spite of the inadequate understanding of the reactions which lead to uterine bleeding, experimental data are available which indicate, in part, the combinations of estrogen and progesterone stimuli which promote bleeding:

1. Bleeding does not occur when estrogen is administered in subthreshold amounts and then withheld (34*).

2. With adequate estrogen administration, bleeding occurs six to ten days after the hormone is withheld or reduced by 50 per cent (34*).

3. Cyclic bleeding is occasionally observed when estrogen is given continuously for long periods of time (57).

4. Prolonged and noncyclic bleeding may be provoked by the administration of large amounts of estrogen (58).

5. Bleeding occurs two to three days after the withdrawal of both estrogen and progesterone (58, 59, 60).

6. Bleeding occurs in spite of continued administration of estrogens and progesterone in amounts adequate to develop progravid endometrial changes (58).

7. Bleeding may occur when progesterone injections are introduced for a short period during the continuous administration of estrogens (58, 61).

8. Bleeding may be temporarily postponed if progesterone is introduced simultaneously with the withdrawal or diminution of estrogen which had previously been administered in constant amounts (58).

9. The capacity of progesterone to induce bleeding in the absence of previous or concomitant estrogen stimulation of the endometrium is still unsettled (49).

These observations indicate that, in general, estrogenic hormone can provoke uterine bleeding in the absence of detectable progesterone. Bleeding from an estrogen-stimulated endometrium may be influenced by progesterone. The combinations and permutations of the mode of administration of these hormones add complexity to the problem. The incomplete correlation of these experimental studies with clinical observations in uterine bleeding gives evidence of the empiricism which accompanies the applications of progesterone therapy in menstrual disorders.

The experiments of Fraenkel (1) have been extended to evaluate the contribution of the corpus luteum hormone to pregnancy in addition to the uterine changes already mentioned. Phelps has observed that the areas of the endometrium which exhibit marked hyperemia are the most frequent sites of ovum implantation. In addition, the coiled arteries reach

maximal development and the endometrial glands exhibit marked secretory activity in these areas (53). Reynolds observed that the endometrial architecture after progesterone stimulation appears to provide a ready access of the trophoblast to the maternal blood supply for nutritional as well as mechanical support. The coiled arteries seem capable of marked elongation and stretching (45*).

The fertilized ova of rabbits develop for several days in the uterine horns before implantation occurs in the progravid endometrium. If the animals are castrated shortly after mating, the fertilized ova do not develop beyond the blastocyst stage. Degeneration and failure of implantation follow (62). Corner concluded that the absence of progestational proliferation might be a cause for failure of implantation (62). Pincus and Enzmann (63*) observed that similar results could be obtained with intact rabbits if relatively large amounts of estrogen were given shortly after mating. The administration of crystalline progesterone permitted implantation of the fertilized ova of castrated rabbits as well as continuation of pregnancy to term (64, 65).

These demonstrations support the concept that progesterone has an important function in preparing the organism for pregnancy, in maintaining the unimplanted ovum, and permitting the continuation of pregnancy. It has not been demonstrated whether progesterone maintains the fertilized human ovum prior to nidation by some direct action or by promoting secretion of nutrients by the proximal portion of the Fallopian tubes (66). Hertig and Rock have recovered young human ova (67). It would be important to determine whether the morphologic abnormalities observed were associated with disturbances in progesterone secretion.

As pregnancy progresses, the uterus enlarges to accommodate the products of conception. The distending action of the pregnancy stimulates growth of the uterus by hypertrophy and hyperplasia of muscle fibers. Reynolds has observed that progesterone administration decreases the tonus of the myometrium and thereby permits passive distention of the uterus. In addition, under the influence of progesterone, greater distention is possible with less growth of the uterine muscle (45*). Uterine irritability and contractions increase when the physiologic limits of uterine distention are approached. Reynolds suggests that this may be one of the multiple factors involved in the initiation of labor (45*).

Heckel and Allen were able to postpone the expected parturition of pregnant rabbits by the continuous administration of progesterone (68). Labor and the attempted delivery of the postmature fetuses was observed when the hormone was discontinued. It has been postulated that the decrease in progesterone secretion toward the end of pregnancy releases the inhibition of oxytocic factors and thus permits the onset of labor.

The excretion of pregnanediol decreases sharply in the few weeks prior to parturition. Similar falls in pregnanediol output occur in the middle trimester of pregnancy without initiating labor. It appears from this consideration that the relative rate of progesterone secretion does not have a profound effect in provoking labor (69).

Removal of the corpora lutea in early pregnancy leads to resorption of embryos of many laboratory animals. The excision of these organs in the latter half of gestation does not result in abortion in all species (45*). It has been concluded that the corpus luteum is not essential for the maintenance of the entire course of pregnancy in all species. In the human, removal of the corpus luteum of early pregnancy does not always lead to abortion (12a, 70, 71, 72). Pratt's report indicates that removal of the corpus luteum in the twenty-first day of a menstrual cycle did not interfere with an unrecognized pregnancy (12). Ask-Upmark noted that abortion did not occur in many patients whose corpora lutea were removed in early pregnancy (70). These studies indicate that extraluteal factors may have a significant function in maintaining human pregnancy in its early stages.

The corpus luteum regresses after the third month of human pregnancy. The placenta then assumes the function of secreting the hormones responsible for the maintenance of gestation (73). Young placental tissue secretes gonadotropins (74, 75). Placental extracts demonstrate progestational and estrogenic activity (10, 11). These studies plus the observations noted previously (72) are evidence that the placenta can secrete at an early stage those hormones which appear necessary for the maintenance of gestation.

The steroid regulation of mammary development and lactation is not clear. Initially, it was observed that estrogenic hormone promotes growth of the ductile system and that the corpus luteum principle then develops the lobulo-alveolar portion (76*). Human breast biopsy specimens removed during the various phases of the menstrual cycle supported this concept (77). There are no reports indicating that exogenous hormone administration can produce these results.

Selye reported growth of the alveolar structures of castrated rats after the administration of progesterone alone (78, 79*). The reports simulate the observations made on the effect of progesterone on the unprimed castrated primate endometrium (80).

The recent reports of Folley and co-workers have prompted reexamination of the previous concepts (81). They observed that the administration of large quantities of estrogens to heifers led to the development of the entire mammary structure and subsequently maintained lactation. These studies have not clarified the physiologic mammary changes in the gravid and nongravid state, although they demonstrate the pharmacologic capacity of the hormones employed.

Progesterone exhibits several functions which resemble those of desoxycorticosterone. The administration of crystalline progesterone can maintain adrenalectomized animals (82). It diminishes sodium and chloride excretion and slightly increases the excretion of potassium (83). Unlike adrenal extracts, progesterone fails to promote excretion of water in adrenalectomized rats (84). Progesterone has slight androgenic activity in rats and can maintain spermatogenesis in these animals after hypophysectomy (85). Although desoxycorticosterone-like action of progesterone has beeen observed in the human (86), no androgenic effect has been noted.

SECRETION AND METABOLISM

It was previously mentioned that there is little progestational activity detectable in body fluids and in the organs which presumably elaborate progesterone (11, 12, 87). Only a fraction of the activity of progesterone is observed in the carcass of rats a few hours after the injection of the hormone (88). Although sensitive and specific methods have been developed for progesterone detection (89, 90), the rapid inactivation of progesterone suggests that measurement of the hormone itself may not provide exact information regarding the quantitative aspects of its secretion.

Histochemical analysis of the organs of secretion do not yet distinguish progesterone from the other steroids present (91, 92). The histologic examination of endometrium stimulated by endogenous progesterone is limited by the lag between secretion and response (21, 22). The measurement of metabolic derivatives of progesterone, pregnanediol in particular, has been employed as an estimate for the duration and quantitation of progesterone secretion. Although the excretion of pregnanediol begins after the onset of progesterone secretion and continues for twenty-four to forty-eight hours after the source of progesterone elaboration has been removed, it has provided useful information regarding the characteristics of the secretion of its precursor (93, 94, 95).

Marrian (96) isolated an inactive steroid alcohol from pregnancy urine five years before the identification of progesterone. Butenandt later named this compound pregnanediol (97). He postulated that pregnanediol might be a metabolic product of progesterone due to the similarity of the chemical structure of the compounds and also because progesterone could be synthesized from it in vitro (98, 99*).

In the human, pregnanediol is excreted as the water-soluble complex salt, sodium pregnanediol glucuronidate ("NaPG") (100, 101). Venning's technique was used to identify this complex salt in urine after the administration of progesterone (102, 103). The results were interpreted as proof that pregnanediol is a metabolic excretion product of progesterone in the human (93, 104). This conversion does not occur in all species (105*).

It has been observed that pregnanediol excretion occurs shortly after ovulation in the human and usually persists until one to four days prior to the succeeding menstrual flow (93, 94). The levels of excretion may vary in the postovulative period (93). Progestational endometrial changes have been demonstrated in those cycles in which pregnanediol excretion occurred (94, 106).

When fertilization occurs, pregnanediol excretion persists during the ensuing amenorrhea (94, 107). During the first trimester the output remains relatively constant at approximately the maximal level (10 mg. per twenty-four hours) observed during the menstrual cycle. Pregnanediol excretion increases irregularly during the second trimester. The increasing levels of pregnanediol reach a maximum several weeks before parturition. From this peak, there is an irregular decline in excretion and pregnanediol is not detected in the urine twenty-four to forty-eight hours after delivery (69, 108, 109). This pattern of pregnanediol excretion is generally observed in pregnancy although there are quantitative variations in individual cases.

Pregnanediol has also been found in the urine of patients with virilizing syndromes due to hyperfunction of the adrenal cortex (110, 111). It is presumed that progesterone is the precursor of pregnanediol in these cases. Since it has been observed that desoxycorticosterone (112, 113) and Δ^5 pregnenolone (114) may be metabolized to pregnanediol, the possibility of alternative pregnanediol precursors must be considered in these cases. It would appear, however, that under physiologic conditions, pregnanediol is principally the excretion product of progesterone.

Marrian has questioned the validity of a "constant relationship between urinary pregnanediol excretion and endogenous progesterone production" (115). In his studies he did not observe a constant relationship between administered progesterone and excreted pregnanediol. These results were in accord with those obtained early in the study of the subject (93, 104). Variations in the relative amounts of recovered pregnanediol led Venning and Browne (93) to think that the uterus was necessary for progesterone conversion. Their hypothesis was negated by the demonstration that men can metabolize progesterone to pregnanediol (104). Hepatic and renal function have been suggested as factors modifying progesterone conversion (116, 117).

Examination of various reports (93, 115, 118, 119) indicate that several investigators, using different techniques for pregnanediol measurement, have recovered pregnanediol amounting to approximately 10 per cent of the progesterone administered to men and to nonpregnant women. When the pregnanediol excretion represented more than 25 per cent of the administered progesterone, it was noted that the female subjects had func-

tioning luteal tissue (93, 119). The administration of progesterone in normal pregnancy led to pregnanediol excretions (in excess of control values) which accounted for more than 25 per cent of the corpus luteum hormone given. These data have been interpreted to indicate that the mode of conversion of pregnanediol is influenced by the functioning corpus luteum and placenta (119). Therefore, it would appear that in the nonpregnant preovulative state, the extent of progesterone conversion to pregnanediol amounts to approximately 10 per cent. If these experiments have duplicated the mode of endogenous progesterone conversion, it would seem that normal pregnanediol excretion represents approximately 25 per cent of the hormone secreted during the luteal nonpregnant state and in normal pregnancy (119). These studies need further confirmation to determine whether the conversion changes are physiologic or an adaptation to a prolonged progesterone stimulus (115, 119).

Additional steroids which have chemical structures similar to progesterone and pregnanediol have been isolated from urine (117*). It has been suggested that these compounds are intermediates in progesterone metabolism. Marrian and co-workers demonstrated that Venning's complex ("NaPG") is a mixture of two steroids—sodium pregnanediol glucuronidate and sodium pregnanolone glucuronidate (120, 121). Dorfman later recovered pregnanolone and pregnanediol from the urine of men who had received progesterone (118). Further study is necessary to determine the fate of the molecule, since the known derivatives of progesterone metabolism account for about 50 per cent of the hormone.

CLINICAL APPLICATIONS

The clinical applications of progesterone in disturbances of the menstrual cycle have been directed toward influencing the histologic characteristics of the endometrium, reducing myometrial activity, modifying bleeding, and restoring menstrual rhythmicity. The efficacy of contemporary progesterone therapy may be attributed in part to an increasing understanding of its physiologic properties and in part to empiricism. The therapeutic applications of progesterone should be evaluated in the light of our limited knowledge of the biologic activity of the hormone and the physiology and pathophysiology of the menstrual cycle.

Kaufmann was among the first to report the use of progestin in the amenorrhea of castrate women. He observed that uterine bleeding occurred in these women after they had received estrone and progestin (30). Whether the bleeding was due to the withdrawal of progestin, estrogen, or both was not discussed. It was later demonstrated that bleeding followed the administration of progesterone to women who had amenorrhea for less than two years (123). Menstruation recurred in some patients after

progesterone therapy had been repeated at monthly intervals for several months (123, 124). Bleeding occurred infrequently when progesterone was administered to women who had amenorrhea for more than two years (49, 125).

Zondek studied the endometria of some of the patients with amenorrhea (49). Progesterone was ineffective in patients with atrophic endometria. He observed that progesterone promoted bleeding when the endometrium demonstrated some evidence of estrogenic proliferation and that some of these patients resumed normal cyclic bleeding. Even though the progesterone given was adequate to induce bleeding, no histologic changes were noted in the endometria.

Previously, amenorrhea was treated with estrogen in combination with progesterone (123). Following this type of therapy, bleeding occurred more consistently than when the corpus luteum hormone was used alone. It was thought that the normal sequence of the menstrual cycle and bleeding could be imitated by administering estrogen first, then giving estrogen and progesterone together and finally discontinuing both hormones (123).

These observations indicate that progesterone does not exert any specific function in initiating the bleeding process. As has been noted, bleeding will follow the withdrawal of estrogenic hormone when it is administered in sufficient amounts (34*). The physiologic contributions of progesterone in facilitating the initiation of regular cycles awaits further study. The reports on the use of progesterone in the treatment of amenorrhea, with or without estrogen, afford practical applications of the hormone.

The endometrium may exhibit many phases of normal and abnormal estrogenic and progestational proliferation during excessively prolonged uterine bleeding (126, 127). Bleeding may diminish and then cease when progesterone is effective in treating this symptom. After the cessation of progesterone therapy, the bleeding resumes in two to three days and continues for four to six days (128). Although prolonged bleeding may recur in some patients after a variable period and amenorrhea may persist in others, the resumption of normal menstrual cycles is also noted (129, 130). Progesterone therapy is beneficial in this disorder of menstruation when the endometrium demonstrates some evidence of estrogenic stimulation (130). The rationale of this therapeutic approach was that the hormone can convert the endometrium to the progravid phase. Upon withdrawal of therapy, bleeding would occur as from the secretory endometrium (128). Initial studies indicated that excessive bleeding was checked in the absence of demonstrable histologic changes in the endometrium (131). Jones and Te Linde administered larger amounts of progesterone and demonstrated conversion of hyperplastic endometrium to the secretory stage (130). From these studies, it becomes apparent that the threshold for

influencing bleeding is less than that which is necessary for promoting histologic changes.

Persistent bleeding from atrophic endometria does not respond to progesterone therapy (130). In the process of irregular shedding of the endometrium, pregnanediol excretion persists during the prolonged bleeding period (132). In these cases the endometrium demonstrates a patchy distribution of normal and abnormal estrogenic and progestational patterns. Progesterone has not been recommended for this type of bleeding.

The results of progesterone therapy in prolonged uterine bleeding are as difficult to explain as those observed in amenorrhea. Hypotheses have been drawn from experimental studies in laboratory animals (128, 131). The evidence to substantiate these ideas has not been forthcoming. The failure to understand the action of endocrine and nonendocrine factors in causing normal and prolonged uterine bleeding prevents a more rational approach to the therapy of this abnormality of menstruation.

The application of progesterone therapy in dysmenorrhea stems from Knaus' observation—a decreased uterine contractility after progesterone administration (40). The initial reports indicate that pain could be relieved and prevented after progesterone was given (133, 134, 135, 136). Dysmenorrhea did not recur in some patients who had repeated series of injections (134, 135). As yet, there is no unanimity of opinion as to the value of progesterone in the treatment of dysmenorrhea.

Many groups have studied uterine contractility in normal and dysmenorrheic women in various phases of the menstrual cycle. The differences in their observations have been indicated (42, 43, 44, 46). There is still no agreement as to the nature of uterine contractions in dysmenorrhea nor the cause of pain (44, 45). Nonendocrine as well as endocrine factors are probably contributing to this symptom.

Progesterone is secreted in ovulatory cycles and it is in these cycles that dysmenorrhea is observed (42, 137). Dysmenorrhea does not occur when ovulation is inhibited by the administration of estrogen in the postmenstrual phase of the cycle (137). Differences in the uterine contractile response to pituitrin during the premenstrual phase were observed by Knaus (40). During the peak of progesterone secretion in ovulatory cycles, the uterine response to pituitrin was inhibited. In the premenstrual phase of these cycles, pituitrin caused exaggerated contractions. In the premenstrual phase of anovulatory cycles, he observed no change in contractile response to pituitrin. It remains to be determined, first, whether the observed influences of estrogen and progesterone in conditioning the uterine response to pituitrin are an exaggeration of the normal physiologic pattern and second, whether they constitute the endocrine factors contributing to dysmenorrhea.

It has been demonstrated that the function of progesterone in maintaining the pregnant state begins before implantation of the fertilized ovum (62). In applying this information to clinical problems, Rutherford suggested that the inadequate secretion of progesterone shortly after fertilization might be responsible for the failure of some patients to maintain successive pregnancies (138). He recommended that the prophylactic use of progesterone in these patients should be instituted in the postovulative phase of the fertile cycle (138). Although his results were encouraging, the study cannot be evaluated because of lack of sufficient controls. Progesterone has been administered in cases of habitual abortion as a prophylactic measure after the diagnosis of pregnancy has been established. The results reported have been uniformly satisfactory even though a wide range of progesterone dosage was used (122*). Eastman has indicated that many of the results might be due to chance (139). The specificity of the therapy may be questioned in the absence of a demonstrable deficiency in progesterone secretion in the pregnancies under consideration (140). Javert and co-workers have observed equally satisfactory results after careful study and therapy along nutritional lines (141). Therapy in habitual abortion should be directed toward an objectively identified etiologic factor.

Progesterone has been employed for the treatment of threatened abortion since 1936. Hertig and Livingstone summarized the reports to 1944 (122*). Equally good results were observed using intravenous "progesterone" in saline (0.1 rabbit unit per cc.) and intramuscular progesterone in oil (20 mg. every other day). These data are difficult to evaluate, considering the multiple factors which may contribute to abortion. Hertig and Livingstone have shown that more than 60 per cent of abortuses which they examined had evidence of an ovular defect (122). The etiology of such abnormalities is unknown. There are nonhormonal factors which contribute to threatened abortion, namely, anatomic and mechanical defects. It seems clear that progesterone therapy is not indicated in all cases of threatened abortion.

Browne, Henry and Venning were the first to demonstrate that the excretion of pregnanediol could be correlated with the clinical course of threatened abortion (69). They reported that abortion occurred in those patients who had less than normal pregnanediol excretion and presumably decreased progesterone secretion. Patients who maintained the pregnancy had normal pregnanediol excretion. Since the symptoms of threatened abortion may occur at the time of corpus luteum regression, they suggested that failure of the placenta to secrete sufficient progesterone at this critical period of gestation might be responsible for the bleeding and abortion.

These observations have been confirmed by other investigators (142, 143, 144).

Guterman suggested that the trend of pregnanediol excretion during the symptoms of threatened abortion might be employed to predict the fate of threatened abortion (145, 146). He observed that when the tend of pregnanediol excretion is toward less than normal levels, abortion occurs in 95 per cent of the cases. When the excretion levels remain normal or rise to normal levels from initially low levels, approximately 80 per cent of the pregnancies are retained. Guterman and Tulsky studied patients with threatened abortion. The pregnanediol excretion was measured before and during progesterone therapy (140). They observed that when pregnanediol excretion levels indicated a poor prognosis, more than 95 per cent of the patients aborted, in spite of progesterone therapy. Progesterone was also administered to patients with normal pregnanediol excretion and approximately 80 per cent of the pregnancies in this group were retained. Thus, progesterone in the doses employed (up to 25 mg. per day intramuscularly) was ineffective in preventing abortion. They recommended that the administration of progesterone in amounts (80 to 120 mg. per twenty-four hours) adequate to raise the pregnanediol excretion to normal levels (10 mg. per twenty-four hours) might aid the retention of pregnancies which demonstrate diminished progesterone secretion. The results of this type of therapy are in process of analysis (to be published).

When the pregnanediol excretion amounts to less than 20 per cent of the administered progesterone, abortion occurs. When the conversion of pregnanediol exceeds 25 per cent, pregnancy is maintained (119). It appears that the mode of metabolism of progesterone is related to the function of the hormone in maintaining pregnancy.

The studies mentioned have been concerned with an objective evaluation of the contribution of progesterone to the maintenance of the integrity of gestation. The patterns of gonadotropin and estrogen excretion in threatened abortion follow a pattern similar to that of pregnanediol. Serum protein-bound iodine measurements have been low in some patients with threatened and habitual abortion. Thus, multiple endocrine factors appear to be in flux during the symptoms of threatened abortion. The relationship of these hormonal changes to the preclinical onset of threatened abortion is only one of the conditions which should be determined, to provide a rational approach to the therapy of threatened abortion.

Smith and Smith and co-workers (147*) observed that a decrease in the excretion of pregnanediol and estrogens accompanied by a rise in chorionic gonadotropin may precede the symptoms of toxemia of pregnancy. This

led to the administration of large amounts of estrogen and progesterone in toxemia. Later, stilbestrol was recommended since Smith, Smith and Hurwitz observed a rise in pregnanediol excretion ("NaPG") following its use (148). Although progesterone and estrogens have been used in the treatment of toxemia, they do not yet appear effective after the onset of the symptoms of this abnormality of pregnancy.

The influence of stilbestrol on pregnanediol excretion has not been established. There are investigators who have noted no change or a decrease in free pregnanediol following the administration of large doses of stilbestrol (149, 150). Differences in the techniques for pregnanediol estimation may be responsible for the discrepancies in results.

The lower abdominal pains experienced by some postpartum women have been attributed to uterine contractions. The administration of progestin relieved the pain and caused a decrease in the amplitude of uterine contractions (151, 152). There has been no general application of progesterone therapy for relief of these symptoms up to the present time.

COMPOUNDS SIMULATING PROGESTERONE

Selye has analyzed the biologic activity of numerous steroids and has observed many that have progestational (luteoid) activity (153, 154). Anhydro-hydroxy-progesterone (ethinyltestosterone, pregneninolone, "oral progesterone") is the most active of these in promoting the development of the progravid endometrium in the estrogen-primed rabbit (153). This compound has been used extensively in clinical studies as a substitute for progesterone because it has been observed to behave as a progestogen when administered by mouth (155). There has been no objective demonstration of its effectiveness in the treatment of dysmenorrhea and in the maintenance of pregnancy threatened with abortion (122, 156). The results obtained using anhydro-hydroxy-progesterone in treating menstrual disturbances simulate those obtained when progesterone is used (157). The administration of anhydro-hydroxy-progesterone does not lead to pregnanediol excretion (118).

There is a slight chemical structure difference between progesterone and desoxycorticosterone. Adequate amounts of desoxycorticosterone can develop a secretory endometrium in the estrogen-primed monkey (158). It can maintain the pregnancy of castrated rats (159). Desoxycorticosterone is not employed clinically as a substitute for progesterone in spite of these luteoid functions. It has been observed that desoxycorticosterone is converted to pregnanediol (113).

Testosterone and methyltestosterone can stimulate the differentiation of progravid endometrium (160). Although testosterone has been used clinically in the treatment of prolonged uterine bleeding and in dysmenor-

rhea, it has not been applied therapeutically as a substitute for progesterone.

DISCUSSION

Born's general hypothesis is now a fact by virtue of many demonstrations in the laboratory mammal. In addition to estrogen, the corpus luteum has yielded progesterone, the hormone which seems to duplicate many of the functions of that gland. Progesterone has been applied in disturbances of the menstrual cycle and in human pregnancy on the basis of its observed biologic activity in rodents and primates.

The results of attempts in the human to elicit the functions of progesterone noted in other species have thus far indicated that there are limitations to such a transference. Progesterone, in general, has analogous activities in the various species tested. Studies indicate that physiologically progesterone does not function independently of other endocrine and nonendocrine factors. The therapeutic applications of progesterone should, therefore, be modified and guided by an evaluation of these factors. Failure to appreciate these elements has limited suggested uses of progesterone to empiric trials.

The limits of knowledge and understanding of the physiology of the menstrual cycle and human pregnancy further hamper a rational application of progesterone in clinical disturbances of these physiologic states. Although progesterone has proved of some empiric value in the therapy of amenorrhea, menometrorrhagia, threatened abortion and dysmenorrhea, a proper evaluation awaits further understanding of the basic physiology and pathophysiology of these processes, as well as a greater appreciation of the influence of the metabolism of the hormones on their biologic activity.

These statements are not intended to discourage or deprecate the use of progesterone in clinical medicine. They are meant to serve as a stimulus for all interested in determining the specific indications for progesterone therapy. The same problems apply to the use of other hormones. As understanding increases, it will facilitate the application of these hormones used therapeutically and will provide greater assurance of clinical success.

SUMMARY

Progesterone has been isolated from the corpus luteum and adrenal gland but has not been identified chemically in normal human tissue. Recent studies indicate that this hormone in combination with estrogen can reproduce the endometrial changes observed during the luteal phase of the menstrual cycle. Uterine bleeding is affected by progesterone only after previous estrogenic stimulation of the endometrium. The relation of progesterone to the etiology and relief of dysmenorrhea is still debated. Although much evidence indicates that progesterone is responsible for the

maintenance of pregnancy, the evaluation of the hormone in functional disturbances of pregnancy awaits further study.

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The 1950 Meeting of the Association for the Study of Internal Secretions

The Thirty-Second Annual Meeting of The Association for the Study of Internal Secretions will be held at the Sir Francis Drake Hotel, Friday and Saturday, June 23 and 24, 1950, in San Francisco, California.

The Committee on Local Arrangements is comprised of Dr. Hans Lisser, Chairman, and Drs. Leslie L. Bennett, Roberto F. Escamilla, Minnie B. Goldberg, Gilbert S. Gordan, and Laurance W. Kinsell.

Hotel accommodations will be difficult to secure on short notice; therefore, members are urged to make their reservations at once. All requests must be addressed to: Dr. William Howard Rustad, American Medical Association (Hotel Committee), Room 200, Civic Auditorium, San Francisco, California.

The scientific sessions will be held in the Empire Room of the Sir Francis Drake, and registration will be in the French Room on the same floor. The annual dinner will be held in the Empire Room on Friday, June 23, at 7:30 P.M., preceded by cocktails at 6:30 P.M. in the French Room.

PROGRAM

FRIDAY, JUNE 23, 1950

8:30 A.M. Registration—French Room

I. 9:30 A.M. Empire Room

E. H. Rynearson, presiding

1. The Effect of Hypophysectomy and Growth Hormone on Phosphorus Metabolism. Irving I. Geschwind (by invitation), Choh Hao Li, and Herbert M. Evans (by invitation).

2. Studies on the Effects of Hypophysectomy and Adrenocorticotrophic Hormones on Certain Phosphorus Fractions in the Rat Liver. Irving I. Geschwind (introduced by Choh Hao Li).

3. The Effect of Growth Hormone on Hepatic Catalase, Erythrocyte Volume, and Hemoglobin in Rats. O. H. Gaebler, and James C. Mathies (by invitation).

4. The Effects of Prolonged Perfusion of the Dog's Pancreas with Glucose. Ernest M. Brown, Jr. (by invitation), F. C. Dohan, L. R. Freedman (by invitation), P. DeMoor (by invitation), and F. D. W. Lukens.

5. Action of Steroids on Cerebral Metabolism. R. C. Bentinck (by invitation), Gilbert S. Gordan, John E. Adams (by invitation), L. H. Arnstein (by invitation), and T. B. Leake (by invitation).

6. Hormonal Factors Affecting Avidin Secretion and Biotin Blood Levels in the Chick. Roy Hertz, W. W. Tullner (by invitation), and F. G. Dhyse (by invitation).

7. A Study of the Metabolism of Single Therapeutic Doses (2.0 mg.) of Estrone in Healthy Women in the Preovulatory and Postovulatory Phases of the Menstrual Cycle. Benjamin F. Stimmel, and Clair L. Stealy.

8. A Steroid which Promotes Tissue Growth without Concomitant Genital Activity. Gilbert S. Gordan, Eugene Eisenberg (by invitation), and Henry D. Moon (by invitation).

9. Factors in Wheat Germ Oil Affecting the Sex Organs of Hypophysectomized Rats. Ezra Levin, John F. Burns, A. Kaplan, and V. K. Collins (introduced by Carl R. Moore).

10. The Use of Progesterone in Treatment of Cancer of the Prostate. J. B. Trunnell, B. J. Duffy, Jr. (by invitation), Victor Marshall (by invitation), Willet Whitmore (by invitation), and Helen Q. Woodard (by invitation).

11. Tracer Studies with Labelled Preparations of Anterior Pituitary Hormones: *ACTH*. Martin Sonenberg, Albert S. Keston, and William L. Money (introduced by Rulon W. Rawson).

12. Fate of Intravenously Administered Adrenocorticotrophic Hormone (*ACTH*). John B. Richards (by invitation), Marshall Merkin (by invitation), Chi Ping Cheng (by invitation), and George Sayers.

13. The Effect of *ACTH* upon the Level of 17-Hydroxycorticosterone in the Adrenal Vein Blood of Dogs. Don H. Nelson (by invitation), Hans Reich (by invitation), and L. T. Samuels.

II. 2:00 P.M. Empire Room

E. A. Doisy, presiding

14. The Functional Relationship between the Pituitary Gland and the Parathyroids. Nils Tornblom (introduced by Fuller Albright).

15. Radioiodine (I^{131}) and Gonadal Function: An Experimental and Clinical Study. B. J. Duffy, Jr. (by invitation), J. T. Godwin (by invitation), J. B. Trunnell, and R. W. Rawson.

16. Carrier-Free Radioactive Iodine (I^{131}) Thyroid Uptake and Urinary Excretion in Normal and Hypothyroid Children. Wm. A. Reilly, and Dina L. Bayer (introduced by H. Lisser).

17. Uptake of Radioactive Iodine (I^{131}) by the Thyroid Gland after Intravenous Administration of Tracer Doses. Joseph P. Kriss.

18. Observations on the Relationship between Bone Marrow Activity and Thyroid Function. Arnold R. Axelrod, and Lawrence Berman (introduced by William O. Maddock).

19. Thyroxine-Like Activity of Acrylic Acid Analogues of Thyroxine. S. B. Barker, H. M. Klitgaard (by invitation), H. B. Dirks, Jr. (by invitation), S. Wawzonek (by invitation), and S. C. Wang (by invitation).

20. Clinical Application of Certain Mercaptoimidazoles to Hyperthyroidism. Thomas

H. McGavack, Jacqueline Chevalley (by invitation), A. M. Shearman (by invitation), I. J. Drekter, and Sidney Stern (by invitation).

21. Thyroid Hormone and Tissue Cholesterol Distribution. Walter Marx, Lore Marx, and Samuel B. Weiss (introduced by H. J. Duel, Jr.).

22. Studies on Hormonal Control of Serum Lipid Partition in Man. David Adlersberg, Louis E. Schaefer (by invitation), and Rhoda Dritch (by invitation).

23. The Effects of *ACTH*, of Cortisone, and of Other Steroid Compounds upon Fat Metabolism in Diabetic and Non-diabetic Human Subjects. Laurance Kinsell, Sheldon Margen, George Michaels (by invitation), Robert Reiss (by invitation), Robert Frantz (by invitation), and John Carbone (by invitation). With the technical assistance of Gerald Liebert, Carl Anderson, Lila Suiter, and Maxine E. Hutchin.

24. The Anabolic Effects of Testosterone Compound as Manifested by Increased Height and Weight Gain in Boys. H. Lisser, and Gilbert S. Gordan.

25. The Effect of Testosterone Administration upon the Human Testis. Carl G. Heller, Warren O. Nelson, Irvin C. Hill (by invitation), Edward Henderson, William O. Maddock, and E. C. Jungck.

26. Sex-Linked Growth Retardation and Juvenile Diabetes Mellitus. Robert R. Commons (by invitation), Oner C. Barker (by invitation), and E. Kost Shelton.

27. Pituitary Infantilism. Treatment with Purified Growth Hormone, Thyroid, and Sublingual Methyl Testosterone. Roberto F. Escamilla, and Leslie Bennett.

III. Annual Dinner, Friday, June 23, 1950

6:30 P.M., Cocktails, French Room
7:30 P.M., Dinner, Empire Room
President E. A. Doisy, presiding

PRESENTATION OF: E. R. Squibb & Sons Award for 1950, Ciba Award for 1950, Ayerst, McKenna & Harrison Fellowship for 1950, Schering Fellowship for 1950.
By *E. C. Kendall*, Chairman of Awards Committee for 1949–50

PRESIDENT'S ADDRESS: DR. DOISY.

SATURDAY, JUNE 24, 1950

IV. 9:00 A.M. Empire Room

J. S. L. Browne, presiding

28. Familial Female Pseudohermaphroditism. R. C. Bentinck (by invitation), H Lisser, Frank Hinman (by invitation), and Herbert C. Traut (by invitation).

29. The Effects on the Linear Growth, Weight Gain, and Histologic Pattern of the Testes of Young Rats by Large Doses of *DOCA*, Lipoadrenal Cortex, Thyroid, Thyroxin, Testosterone Propionate and Physiologic Doses of Testosterone Propionate. Morton J. Nyda and Gerson R. Biskind.

30. The Relation of Adrenal Activity to Experimental Hypertension. Lena A. Lewis, and Irvin H. Page.

31. Interrelations between the Adrenal Cortex and Lymphoidal Tissues: Growth of Adrenal Transplants in Spleen and Thymus with Local Thymic Atrophy. Edward J. Bomze (by invitation), and Nathan B. Friedman.

32. The Effect of Adrenal Transplanting on the Stress Reaction to Hypoxia. L. L. Langley (introduced by Robert S. Teague).

33. The Use of Cystine Labeled with S^{35} as a Tool for Studying Adrenal Physiology and Protein Metabolism. Norman D. Lee (by invitation), and Robert H. Williams.

34. Changes in Responsiveness to Insulin in Diabetes Mellitus Induced by the Administration of Desoxycorticosterone. Hyman J. Zimmerman (by invitation), Alvin E. Parish (by invitation), and Louis K. Alpert.

35. The Metabolic Effects of *ACTH* in a Patient with Cushing's Syndrome and Acromegaly. Leonard P. Eliel, and Olof H. Pearson (introduced by Konrad Dobriner).

36. Effect of Adrenocorticotrophic Hormone (*ACTH*) on the Pathological Changes Induced by Desoxycorticosterone Acetate (*DCA*). Charles A. Rosenberg (by invitation), Dixon M. Woodbury (by invitation), and George Sayers.

37. The Response of the Adrenal Cortex and Thyroid Gland and Cortisone in Patients with Hypothyroidism and the Nephrotic Syndrome. S. R. Hill, Jr. (by invitation), P. H. Forsham (by invitation), M. Roche (by invitation), and G. W. Thorn.

38. The Effect of Small Dosages of *ACTH* Protein and *ACTH* Peptides in Cases of Rheumatoid Arthritis. Rolf Luft, Bjorn Sjogren (by invitation), and Choh Hao Li.

39. Erythrocyte Sedimentation Rate in DCA and Cortisone Treated Animals. Hans Selye, and Niall Carey (by invitation).

40. Oral Steroid Administration in Rheumatoid Arthritis. Harry Freeman (by invitation), and Gregory Pincus.

Conjoined Program with
American Diabetes Association

V. 2:00 P.M. Empire Room

Howard F. Root and E. A. Doisy, presiding

41. Growth Hormone and Experimental Diabetes. F. G. Young.

42. *ACTH* Diabetes in Man. The End Organ Response Versus the Adrenocortical Response. Jerome W. Conn.

43. Studies on Carbohydrate Metabolism in Adrenal Cortical Hypersecretion. P. H. Forsham (by invitation), G. W. Thorn, T. F. Frawley (by invitation), and D. L. Wilson (by invitation).

44. Modification of the Metabolic Effects of Adrenocorticotrophic Hormone and Growth Hormone by the Administration of a Diet High in Potassium Chloride. Leslie L. Bennett, John E. Whitney (by invitation), and Constantine Glafkides (by invitation).

45. Observations on the Pathogenesis of Cushing's Syndrome. Randall G. Sprague, Harold L. Mason, and Marschelle H. Power (by invitation).

46. Pathways of Corticosterone Synthesis. O. Hechter, B. P. Jacobsen (by invitation), R. Jeanloz (by invitation), H. Levy (by invitation), G. Pincus, and V. Schenker (by invitation).

47. The Islets of Langerhans in Man Visualized by Phase Contrast Microscopy. W. Stanley Hartroft (by invitation).

48. The Incidence and Forms of Vascular Disease in Diabetes. E. T. Bell (by invitation).

4:00–4:30 P.M.—General Discussion

VI. 4:30 P.M. Annual Business Meeting

TO BE READ BY TITLE

49. The Effect of Desoxycorticosterone Acetate (*DCA*) on Blood Pressure and Renal Function in Human Subjects. Rolf Luft, and Bjorn Sjogren (by invitation).

50. Studies on Circulating Growth Hormone. Francis S. Greenspan (introduced by Choh Hao Li).

51. Estrogenic Interruption of Broodiness in the Fowl. R. George Jaap, and Edward F. Godrey (by invitation).

52. Response to Estrone of Intraocular Endometrial Implants of Littermates in Female Rabbits with Uteri Intact. Elly M. Jacobsen (introduced by Clara M. Szego).

53. Quantitative Studies on the Production of Pseudopregnancy by Estrogens. Albert Segaloff.

54. The Agreement of Clinical Diagnosis with Vaginal Smears, as Shown by the Evaluation of Symptoms, Colpocytogram, and Glycogen Index. Manual M. Villaverde.

55. The Effect of Steroid Hormones Applied Directly to Senile Human Skin. Joseph W. Goldzieher, William B. Rawls (by invitation), Irene S. Roberts (by invitation), and Max A. Goldzieher.

56. Oral Administration of Wheat Germ Oil Concentrate as an Aid in the Reduction of Pregnancy Complications. Wynne M. Silbernagel, and James B. Patterson (by invitation).

57. The Role of the Liver and Kidney in the Metabolism of Intravenous Testosterone by Human Subjects. C. D. West (by invitation), Harold Brown (by invitation), Frank H. Tyler, and L. T. Samuels.

58. Frequency of Retarded Bone Age in a Group of Enuretic Children. R. M. Archibald.

59. Clinical Experience with the Use of Cortisone Pellets in the Treatment of Adrenal Cortical Insufficiency. G. W. Thorn, P. H. Forsham (by invitation), T. F. Frawley (by invitation), and S. R. Hill, Jr. (by invitation).

60. A Study of Adrenal Cortical Response in Health and Disease. The 48-Hour *ACTH* Test. M. Roche (by invitation), P. H. Forsham (by invitation), C. C. Forsham (by invitation), and G. W. Thorn.

61. The Effects of *ACTH*, and of Steroid Compounds upon Ketolysis in Diabetic and Non-Diabetic Human Subjects. Sheldon Margen, George Michaels (by invitation), Charles Mudrick (by invitation), Harry Barton (by invitation), Robert Reiss, and Laurance Kinsell. With the technical assistance of Gerald Liebert, Carl Anderson, Lila Suiter, Maxine Hutchin, and Don Myers.

62. Administration and Subsequent Withdrawal of *ACTH* Studied Simultaneously in a Gouty Father and in His Hyperuricemic Son. W. Q. Wolfson, C. Cohn, C. Shore (by invitation), W. Wise (by invitation), and S. Sideman (by invitation).

63. Increased Adrenal Cortical Function Accompanying Prolonged Remission Induced by *ACTH* in Myasthenia Gravis. B. Katz (by invitation), M. Katz (by invitation), W. Q. Wolfson, C. Cohn, and B. B. Rubenstein.

64. Adactar and Adactar-Gelatins Pituitary Adrenocorticotrophin Preparations with Prolonged Activity. W. Q. Wolfson, R. E. Thompson, and C. Cohn.

65. The Effect of Crude Anterior Pituitary Preparation on Haemopoiesis in Normal, Thyroidectomized and Gonadectomized Rats. Niall Carey (introduced by Hans Selye).

66. Thyroid Extract Versus Thyroxine. Critical Evaluation with Particular Reference to Functional Infertility. A. R. Abarbanel.

67. Thyrotoxin Neuropathy. Max A. Goldzieher, Thomas H. McGavack, and Joseph W. Goldzieher.

68. The Treatment with Desiccated Thyroid of Periodic Disturbances with Menses in Mentally Defective Patients. Meyer M. Harris, and Theodora M. Abel (by invitation).

69. Hypopituitary Myxedema, A Non-Cachectic Form of Simmonds' Disease: Report of Five Cases Emphasizing the Significance of Plasma Cholesterol Levels. Leela S. Craig, Roberto F. Escamilla, and Gilbert S. Gordan.

70. Cushing's Syndrome from Adrenal Cortical Tumor with Special Reference to the Surgical Mortality: A Report of Three Cases and Review of the Literature. M. B. Goldberg, Gilbert S. Gordan, William C. Deamer (by invitation), Frank Hinman, Jr. (by invitation), and Elliot Rapaport (by invitation).

71. Growth and Maturation in a Case of Idiopathic Precocious Puberty. Leona M. Bayer.

72. Familial Insulin-Resistant Diabetes Mellitus. Myron S. Arrick (introduced by E. A. Doisy).

73. Adjuvant Use of Pregnenolone in Seminal Inadequacy. A. R. Abarbanel.

74. A Simple Method for the Determination of Neutral 17-Ketosteroids Based on a Novel Means for the Removal of Interfering Pigments. I. J. Drekter, Alexander Heisler (by invitation), and Thomas H. McGavack.

75. Studies with Pregnenolone: 1. Nitrogen and Electrolytic Balance. Jonas Weissberg (by invitation), Jacqueline Chevalley (by invitation), and Thomas H. McGavack.

76. Studies with Pregnenolone: 2. Clinical Applications. Thomas M. McGavack, Jacqueline Chevalley (by invitation), and Jonas Weissberg (by invitation).

77. The Effect of Female Hormones on the Basal Body Temperature. M. James Whitelaw.

78. The Treatment of Juvenile Acne with Topical Estrogens. M. James Whitelaw.

79. Acetoxypregnenolone. George Joyce Hall.

GRADUATE COURSE IN ENDOCRINOLOGY MEDICAL COLLEGE OF GEORGIA

The Medical College of Georgia announces the 7th Graduate Course in Endocrinology which will be given September 4–9, inclusive. The course is offered as a refresher and guide in those aspects of basic endocrinology which have practical and clinical application. The lectures will deal with the endocrine problems which arise in everyday practice and are designed for the practicing physician. Registration is limited to fifty. Enrollment is open to all qualified physicians. Applications should be addressed to the Registrar, Medical College of Georgia, Augusta, Ga.

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BOOKS RECEIVED

THE following list acknowledges receipt of books which it has not yet been possible for us to review:

Endokrinologie und Praxis: der endokrine Status. By PROF. DR. HERMANN BERNHARDT, ärztlicher Direktor des Städt. Krankenhauses Berlin-Spandau (Reihe, innere Medizin; Herausgeber, Dozent DR. HANS-JÜRGEN OETTEL, Berlin); 92 pages, 1949. Berliner medizinische Verlagsanstalt GMBH, Berlin.

Primary Carcinoma of the Vagina. By ROBERT G. LIVINGSTONE, M.D., formerly member Resident Staff, Gynecological Division, Memorial Hospital for Treatment of Cancer and Allied Diseases, New York City: a monograph in American Lectures in Obstetrics and Gynecology, edited by JOHN L. MCKELVEY, M.D.; 82 pages; 8 tables; 2 illustrations; 1950. Charles C Thomas. Publisher, Springfield, Illinois. Price $2.00.

Textbook of Endocrinology. Edited by ROBERT H. WILLIAMS, M.D., Executive Officer and Professor of Medicine, University of Washington Medical School, Seattle, with the collaboration of DRS. PETER H. FORSHAM, HARRY B. FRIEDGOOD JOHN EAGER HOWARD, EDWIN J. KEPLER, WILLIAM LOCKE, L. HARRY NEWBURGH, EDWARD C. REIFENSTEIN, JR., WILLIAM W. SCOTT, GEORGE VAN S. SMITH, GEORGE W. THORN and LAWSON WILKINS; 805 pages; 168 illustrations; 60 tables; 1950. W. B. Saunders Company, West Washington Square, Philadelphia.

Transactions of the American Goiter Association. 1949 Annual Session, May 26–28, Madison, Wisconsin; 476 pages; 55 tables; 115 illustrations; 1950. Charles C Thomas. Publisher, Springfield, Illinois. Price $10.50.